GENETICS

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Effective with Volume 45, 1960

1. GENETICS will be changed from a bimonthly to a monthly journal.

2. The annual volume of the journal will include a minimum of 1,000 pages. The Editors expect to include enough additional pages to print all acceptable manuscripts if funds are available.

3. The subscription rate will be $12.00 for a complete volume (January-December). Foreign postage will be $1.00 extra. Single copies will cost $1.50.

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An author is expected to submit to the Editors two typewritten copies of a manuscript, including an original typed copy. Manuscripts must be double spaced throughout, including the footnotes, tabular material, legends and Literature Cited.

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GENE CONVERSION OF CYSTEINE MUTANTS IN NEUROSPORA[1]

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SEVERAL cases of the following type have been reported in studies of microorganisms in recent years: two or more mutants of independent origin have similar phenotypes and appear to be allelic to each other or very closely linked; crosses between the mutants yield a small proportion of nonmutant progeny, but the event producing these recombinants does not have the characteristics of a crossover. Such a case, involving two cysteine-requiring mutants of *Neurospora crassa*, is reported here.

METHODS

Crosses were made at 25°C on synthetic crossing medium (WESTERGAARD and MITCHELL 1947) supplemented with the growth factors required by the protoperithecial parent (250 mg/liter of cysteine and in some cases 50 mg/liter of adenine or of lysine). To isolate cysteine-independent progeny, ascospores were picked up from the wall of the cross tube in loopfuls of water and spread on petri plates of Fries minimal medium (BEADLE and TATUM 1945) without cysteine, but supplemented to be nonselective for linked markers; most plates contained less than 500 ripe spores. Plates were heat shocked and then incubated at 25°C. Starting 15 hours after heat shock, plates were examined at intervals for the next 24 hours, and all cysteine-independent colonies were transferred to slants of complete medium to classify for the linked markers. Total numbers of germinated spores were determined by direct counts on the same plates.

Forty-two of the cysteine-independent strains were crossed to wild type to confirm that their genotypes corresponded to the vegetative phenotypes. Analyses of asci from these crosses revealed in every case that vegetative classification had been correct. This was a check against misclassification of linked markers and also against the possibility of the *cys+* condition arising from a pseudo-wild type (MITCHELL, PITTENGER and MITCHELL 1952) rather than a true *cys+* genetic region.

EXPERIMENTAL RESULTS

Strains 80702 and 48401 are single gene cysteine-requiring mutants of Neurospora. They differ in nutritional responses at 25°C. 80702 (hereafter referred to as *cys-t*) will grow on minimal medium supplemented with either thiosulfate or cysteine, while 48401 (*cys-c*) will grow on cysteine but not on thiosulfate. (At

[1] This investigation was supported by a grant from the National Science Foundation.

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35°C both mutants respond slightly to thiosulfate.) These mutants do not appear to complement each other in heterocaryons. A cysteine-independent heterocaryon made up of strains carrying the two mutants has not been obtained. A heterocaryon of such strains, "forced" by other auxotrophic mutants present in the component strains, required cysteine for growth.

Both mutants are known to be in linkage group VI (M. FLING, unpublished). A cross between them yields about 0.2 percent cysteine-independent progeny. Crosses of *cys-c* × *cys-c* or *cys-t* × *cys-t* yield no *cys*+ progeny. If the *cys*+ spores from the cross between the two mutants resulted from crossing over, it would mean that the two cysteine loci were 0.4 map units apart. However, the *cys*+ progeny appear to result not from crossing over, but rather from gene conversion (MITCHELL 1955). (In this report, the term "gene conversion" is used to mean recombination between closely linked or allelic mutant genes by a process not having the characteristics of crossing over; the use of the term does not imply any specific mechanism for the event.)

Evidence that cys+ *spores do not result from crossing over:* The cysteine region of group VI lies between the loci of the mutants *un* and *ylo* (STADLER 1956). *un* is a temperature mutant which grows on minimal medium at 25°C but has an unknown requirement at 35°C; *ylo* is a visible mutant with yellow conidia instead of the wild type orange. The cross *un cys-c ylo*+ × *un*+ *cys-t ylo* yielded 137 *cys*+ progeny among 72,545 germinated spores. The total recombination frequency between *un* and *ylo* in this cross was 7.7 percent. If the cysteine-independent spores had arisen by crossing over between two separate cysteine loci, the majority of them might have been expected to be one recombinant type for the outside markers (either *un cys*+ *ylo* or *un*+ *cys*+ *ylo*+). Instead, all four possible combinations of outside markers occurred with appreciable frequencies among the *cys*+ progeny (Table 1).

Asci of the above cross were dissected into spore pairs and examined to determine whether the event yielding a *cys*+ spore pair also gave a complementary product (a cysteine double mutant) as would be expected from crossing over. A total of 153 asci were dissected in which all four spore pairs germinated; these included three asci each containing one *cys*+ spore pair (Table 2). The nine cysteine-requiring strains from these asci were all checked for the double mutant

TABLE 1

Cys+ *progeny of the cross* un cys-c ylo+ A × un+ cys-t ylo a

Crossover map distances: *un* 2.1 *cys* 5.6 *ylo*
Cys+ spores among 72,545 germinated random spores:

68	*un cys*+ *ylo*+
26	*un*+ *cys*+ *ylo*
18	*un cys*+ *ylo*
25	*un*+ *cys*+ *ylo*+
137	TOTAL

TABLE 2

Asci containing cys+ *progeny*

un	*cys**	*ylo*	Mating type	Backcross test (Production of *cys*+ progeny in cross to) *cys-c*	*cys-t*
+	+	+	a		
un	*cys-c*	+	a	—	+
un	*cys-t*	*ylo*	A	+	—
+	*cys-t*	*ylo*	A	+	—
un	*cys-c*	+	A	—	+
un	*cys-c*	+	A	—	+
+	*cys-t*	*ylo*	a	+	—
+	+	*ylo*	a		
+	+	*ylo*	A		
+	*cys-t*	*ylo*	A	+	—
un	*cys-c*	+	a	—	+
un	*cys-c*	+	a	—	+

* The *cys* classifications in this column are based on nutritional tests at 25°C.

condition by the backcross test of MITCHELL (1955). (The basis of this test is the assumption that a cysteine double mutant will fail to give *cys*+ convertants in crosses to either parental mutant). Each of the nine proved to be one or the other of the parental single mutant types (Table 2).

Relationship of gene conversion to crossing over: The event yielding *cys*+ progeny does not have the characteristics of classical crossing over, but it does show a correlation with recombination between *un* and *ylo*. Only 7.7 percent of the spores in the general population are recombinants for *un* and *ylo*, while 43 of the 137 *cys*+ spores are recombinants for these markers (Table 1).

In Neurospora, different crosses segregating for the same markers frequently show pronounced differences in recombination frequency (STADLER 1956). Four more crosses of *cys-c* × *cys-t* have been studied in which *un* and *ylo* were also segregating. The aim was to learn whether changes in the frequency of crossing over would result in any consistent change in the frequency or type of *cys*+ conversion.

In Table 3 the five *cys-c* × *cys-t* crosses are arranged in order of increasing recombination frequency for the *un-cys-ylo* region. From cross 1 to cross 5 there is a threefold increase in recombination. There is no corresponding increase in conversion frequency; in fact, the total frequency of conversion to *cys*+ shows only slight changes in any of the five crosses.

When the *cys*+ progeny are separated into the four different outside marker combinations, it is seen that the parental combination of markers which came into the cross with *cys-c* is consistently more frequent than the *cys-t* marker combination. Among *cys*+ progeny with nonparental combinations of *un* and *ylo*, those with the left-hand marker from the *cys-t* parent and the right-hand marker from *cys-c* are consistently more frequent than the reverse combination. But the

TABLE 3

Crossing over and gene conversion in the cys *region*

—*lys*—*un*—*cys*—*ylo*—*ad*—centromere—

Cross	Map distance†				Cys+ per 10^4 germinated spores* (actual numbers in brackets)				
	lys -un	*un- cys*	*cys- ylo*	*ylo -ad*	c+c	t+t	c+t	t+c	Total
1	..	2.8	3.8	3.8	16.4 (40)	1.6 (4)	0.4 (1)	10.0 (25)	28.6 (70 in 24,435)
2	..	2.1	5.6	..	9.4 (68)	3.6 (26)	2.5 (18)	3.4 (25)	18.9 (137 in 72,545)
3	1.2	4.9	6.2	..	13.6 (28)	1.4 (3)	2.9 (6)	5.8 (12)	23.7 (49 in 20,634)
4	..	8.1	13.0	10.8	6.8 (25)	4.1 (15)	0.8 (3)	6.2 (23)	17.9 (66 in 36,781)
5	..	5.1	17.5	10.7	9.0 (29)	2.5 (8)	0.3 (1)	10.6 (34)	22.4 (72 in 32,059)

cross 1: *un cys-c ylo+ ad+ 7A* × *un+ cys-t ylo ad* 13*a*
cross 2: *un cys-c ylo+ A* × *un+ cys-t ylo a*
cross 3: *lys+ un cys-c ylo A* × *lys un+ cys-t ylo+ a*
cross 4: *un cys-c ylo+ ad+ A* × *un+ cys-t ylo ad* 3*a*
cross 5: *un cys-c ylo+ ad+ A* × *un+ cys-t ylo ad* 32*a*

* *cys+* progeny are here classed according to which of the parent strains provided the linked markers immediately around the cysteine locus. For example, "c + t" designates all *cys+* spores which carry the left-hand marker from the *cys-c* parent and the right-hand marker from the *cys-t* parent.
† Map distances are based on the classification of about 200 random spores per cross.

fluctuations of these frequencies show no apparent correlation to recombination frequency.

The length of the region of interaction: While conversion to *cys+* shows a correlation with recombination for neighboring markers, it is not the product of a simple reciprocal exchange between two separate cysteine loci; thus the recombination events taking place in this region must be more complex. It would be of interest to know whether these coincident recombination events take place at a single point or whether the events extend any detectable distance along the chromosomes. Let us call that localized section of the chromosome pair which may be the site of recombination events (either conversion or crossing over or both) the "region of interaction". We should like to determine the length of this region.

In crosses 1, 3, 4, and 5 there are two segregating markers on the same side of *cys*. (The lysine mutant in cross 3, *DS6-85*, is allelic or closely linked to *asco*, a lysine-requiring mutant which arrests ascospore maturation; spores carrying *DS6-85* mature normally. The adenine mutant in crosses 1, 4, and 5 is the one known as *ad-1*.) These crosses offer a method of measuring the length of the region of interaction.

If linked markers A and B are segregating in a cross between the two cysteine mutants, and if the order of the loci is A-B-*cys*, we may be able to compare the length of the region of interaction with the B-*cys* distance. We will compare the

A-B recombination frequency among the general population to that among *cys*+ convertants. If a region of interaction were too short to account for both events, A-B recombinants should be no more frequent among *cys*+ convertants than among random spores (and perhaps less frequent, as a result of chromosome interference). If the region of interaction were long enough to encompass the B-*cys* distance, we might expect a higher frequency of A-B recombinants among convertants than among random spores.

The frequency of *ylo-ad* recombination among *cys*+ convertants in crosses 1, 4, and 5 is not higher than the frequency of *ylo-ad* recombination in the general population (Table 4). There is thus no evidence that regions of interaction in these crosses are long enough to include the *cys-ylo* interval. Likewise, the results from cross 3 do not indicate that the region of interaction is longer than the *un-cys* distance. A similar result was reported by FREESE (1957a) in crosses between para-aminobenzoic acid requiring mutants of Neurospora. There was no correlation between conversion to *pab*+ and crossing over in nearby marked regions.

DISCUSSION

A correlation between gene conversion and recombination for linked markers has been demonstrated in several studies (for example: MITCHELL 1955; ST. LAWRENCE 1956; FREESE 1957a, b; CASE and GILES 1958; ROMAN and JACOB 1958), and two types of hypotheses have emerged regarding the relationship of gene conversion to crossing over:

TABLE 4

Relationship of cys+ *conversion to recombination in region linked to* cys *but not adjacent to it*

—*lys*—*un*—*cys*—*ylo*—*ad*—centromere—			
	un cys-c ylo+ *ad*+ × *un*+ *cys-t ylo ad*		
Cross	*cys-ylo* recombination	*ylo-ad* recombination in general population	*ylo-ad* recombination among *cys*+ convertants
1	2.8%	8/212 (3.8%)	2/70 (1 + + + *ad*, 1 *un* + *ylo* +)
4	8.1%	20/185 (10.8%)	5/66 (3 + + + *ad*, 1 *un* + *ylo* +, 1 + + *ylo* +)
5	5.1%	19/177 (10.7%)	1/72 (+ + + *ad*)
	lys+ *un cys-c ylo* × *lys un*+ *cys-t ylo*+		
Cross	*un-cys* recombination	*lys-un* recombination in general population	*lys-un* recombination among *cys*+ convertants
3	4.9%	2/162 (1.2%)	1/49 (*lys un* + *ylo*)

Separate events: MITCHELL (1955) proposed that crossing over and conversion were separate events but that both might be favored by an especially intimate pairing of the duplicating strands. If the degree of association were not uniform along the length of a pair of homologous chromosomes, then the two types of recombination event would show a correlation in regions of close pairing. It has been suggested (TAYLOR 1957; ROMAN and JACOB 1958) that crossing over might represent exchange along the protein chromosome backbone, while conversion takes place along DNA side chains.

Single event: FREESE (1957b) has proposed a "switch hypothesis" by which the same event may result in both conversion and outside marker recombination. "Within a region of intimate pairing two new chromosomal strands duplicate partially along one parental strand, partially along the other, switching forth and back from one information source to the other." The number of switches may be two or three or more, thus randomizing outside marker combinations. The two strands are not required to make exactly reciprocal switches; this would account for the absence of the complementary product of conversion (Figure 1).

Crosses with markedly different recombination frequencies in the region around the cysteine locus do not show corresponding differences in the frequency of gene conversion. This finding does not lend strong support to either the single

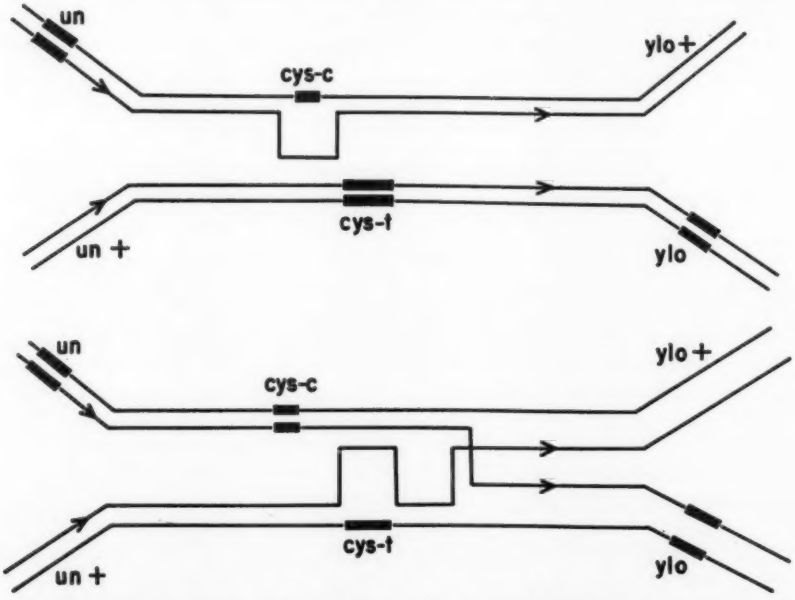

FIGURE 1.—Gene conversion at the cysteine locus by the switch hypothesis. Above: conversion of *cys-c* to *cys* + without linked marker recombination. Below: conversion of *cys-t* to *cys* + with marker recombination.

event or the separate events hypothesis. Perhaps any such "separation" of the two types of recombination argues for their being separate events. However, results somewhat different from these might be predicted from the separate events hypothesis in its simplest form. A constant rate of gene conversion with different recombination frequencies might be expected, but one would predict that increased recombination would shift conversion products with parental combinations of markers to those with nonparental combinations. No such trend is evident from the data.

One aspect of the results which appears to put constraint on the single event hypothesis of FREESE is the finding of a consistent inequality in the numbers of the two types of convertants with parental combinations of adjacent markers; in every cross the *cys-c* combination occurred more frequently than the *cys-t* combination (Table 3). According to the switch hypothesis either of these types could result from a double switch, and there seemed no *a priori* reason to expect the event to embrace one of the mutant genes more often than the other. It might be argued, however, that the length of the mutant region in *cys-t* is similar to the distance between successive switches in a switch region, while the mutant region of *cys-c* is shorter. In such a situation, only a small fraction of the double switches would be so placed as to completely extirpate the mutant region of *cys-t*, while the removal of *cys-c* would be accomplished more often (see Figure 1).

The experiment designed to measure the length of the region of interaction offers no basis of choice between the single event and the separate events hypotheses. The region of interaction by the switching hypothesis would be the region of switching, while the region of interaction for the separate events hypothesis might be the region of especially close pairing. The results observed here serve only to put an upper limit on the length of this region.

Among *cys*+ convertants with nonparental combinations of markers at the *un* and *ylo* loci, all five crosses yielded more with the left-hand marker from the *cys-t* parent and the right-hand marker from the *cys-c* parent than the reverse combination. Such a disparity might be explained in at least two ways:

1) The two cysteine mutant genes might be arranged along the linear axis of the chromosome in the order *un—cys-c—cys-t—ylo*, so that a single exchange could yield a *cys*+ product with the more frequent marker combination, while a more complicated event would be required to yield the reverse combination. Such a linear separation of mutant genes interacting in gene conversion is decisively demonstrated in the study of pantothenic acid mutants of Neurospora of CASE and GILES (1958). A linear arrangement of the mutant regions is implicit in the switch hypothesis, but not necessarily in the separate events scheme (MITCHELL 1955, ROMAN and JACOB 1958).

2) If *cys-c* and *cys-t* were *not* at separate loci along the linear axis of the chromosome, the two classes of convertants with nonparental combinations of markers might still occur in different frequencies, if these three conditions prevailed:

a) gene conversion and crossing over are separate events;

b) *cys-c* and *cys-t* are converted to *cys*+ with different frequencies;

c) the amount of crossing over in the adjacent marked region on the left of *cys* is different from that on the right.

Under these conditions the majority type would be that produced by the more frequent conversion accompanied by the more frequent crossover. In the present crosses the *cys-c* gene appears to be the more frequently converted to *cys*+ (from the numbers of convertants with parental combinations of markers) and the more frequent crossover is in the *cys-ylo* region (Table 3). This combination of more frequent events would yield a *cys*+ convertant with the left-hand marker from the *cys-c* parent and the right-hand marker from the *cys-t* parent. However, this is the class that is consistently *less* frequent in the data than the reverse combination. Therefore, this explanation is untenable for the present case.

It should be pointed out that the *cys-c* parent carries the *un* marker in all five crosses. Viability of strains carrying this marker could be involved in the different frequencies of the marker combinations accompanying conversion. This appears improbable, as the more frequent parental combination is that carrying *un*, while the more frequent nonparental combination is that carrying *un*+. However, a bias due to marker viability can only be ruled out decisively by the study of a cross segregating for the same markers in the opposite alignment.

The author is inclined to the view that conversion is a separate event from crossing over and that the mutant genes participating in conversion (in this case, at least) are arranged along the linear axis of the chromosome pair, possibly as illustrated in Figure 2. *Cys*+ progeny could result from either of two processes. Conversion of either *cys* mutant gene to *cys*+ could take place by a nonreciprocal copying error along the DNA side chain; this process would show a correlation with crossing over in the *un-cys-ylo* region if both processes were favored by the same special pairing conditions. Secondly, *cys*+ progeny could be produced by reciprocal crossing over along the chromosome backbone between the attachment points of the *cys* mutant side chains. Although reciprocal recombination between the *cys* mutants was not observed in the asci analyzed in the present study, such an event has been demonstrated in similar situations in yeast (ROMAN 1958) and Neurospora (CASE and GILES 1958).

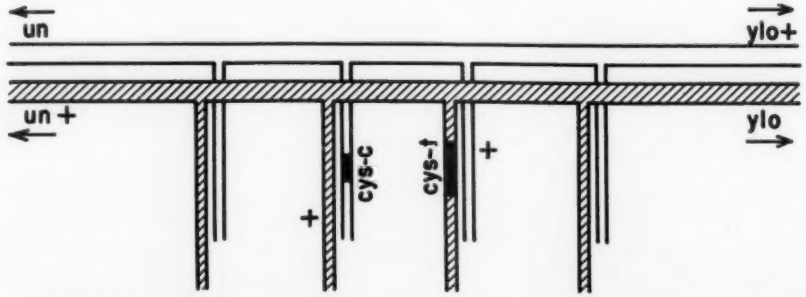

FIGURE 2.—See text for description.

SUMMARY

1. Crosses between two cysteine-requiring strains of Neurospora with mutant genes at the same locus or very closely linked loci yield 0.2 percent cysteine-independent progeny by a process other than crossing over; it is therefore termed "gene conversion."

2. Large changes in the recombination frequency for regions adjacent to the cysteine locus are not accompanied by extensive changes in the rate or pattern of gene conversion at this locus.

3. There is a high coincidence between gene conversion at the cysteine locus and recombination in the marked regions adjacent to this locus. The segment involved in such coincident recombination events appears to be shorter than the distance from the cysteine locus to the closest known marker on either side.

4. Five crosses between the two cysteine mutants have been studied in which linked markers were segregating on both sides. The relative frequencies of the different marker combinations accompanying gene conversion suggest that conversion and crossing over are separate events and that the two cysteine mutants are at separable loci along the chromosome.

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HETEROTIC EFFECTS OF A CHROMOSOMAL SEGMENT IN MAIZE[1]

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THE majority of studies on heterosis are concerned with observations of quantitative traits whose inheritance is complex. Identification and location of the genetic determiners of these traits would appear a desirable but difficult accomplishment. However, a segment-by-segment analysis of the genome to determine the presence or absence of clusters of such genes should bring us a step closer to the genetic mechanisms underlying the phenomenon of heterosis.

A method has been suggested by DOBZHANSKY and RHOADES (1938) in which genes affecting agronomic characters may be located by means of paracentric inversions. In plants heterozygous for such inversions, the crossovers that occur within the inverted segments are only rarely recovered in viable gametes. Thus, the inverted segment is inherited as a unit and all genes contained within the segment are completely linked with very few exceptions. This method is especially advantageous if the agronomic trait being considered is determined by a large number of genes with small effects. In such cases the combined effect of genes located in the inverted segment may permit their detection. In plants without inversions, where crossing over breaks up favorable combinations of minor genes, the existence of such genes is more difficult to demonstrate. By making use of a paracentric inversion with a genetic marker, it is possible to follow a cluster of genes from parent to F_1 and into the backcross or F_2 generations. A comparison is made of plants differing genetically on the average only in the constitution of the specific chromosomal segment, whose length depends on the particular inversion involved. If these plants exhibit significant differences in the characters analyzed, it is assumed that the genes responsible are located in the inverted segment.

SPRAGUE (1941), using *In 5a*, proved this method to be effective. He crossed the *In 5a* strain to a 15-generation inbred dent corn line and the F_1 plants were backcrossed to the inversion strain. Comparisons for agronomic characters were made between plants carrying the heterozygous and the homozygous inverted segments. The heterozygous class was superior to the homozygous class in yield and kernel weight and the differences were significant. No differences were found in plant or ear height, number of ears per plot, kernel row number, or moisture content. Since the experiment of SPRAGUE involved only a single inbred line, a more extensive survey of elite inbred lines was thought to be desirable. The present work consists of an analysis of 15 inbred lines for the presence and location of genes affecting various agronomic characters. Each of these inbred lines was crossed

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with a single paracentric inversion strain, which was used as a standard in comparisons of plants carrying a genetic contribution from the inbred and those possessing the homozygous inversion. It must be emphasized that genetic determiners held in common by both the inversion stock and a particular inbred line will not be detected by this method, even though these genes may be of major importance.

MATERIALS AND METHODS

The paracentric inversion involved in this investigation is *In 3a*, which was studied by RHOADES and DEMPSEY (1953). The inverted segment includes about half the length of the long arm of chromosome 3. The a_1 (colorless aleurone) allele, which is located within the inverted segment, is used as the marker gene for the inversion. The homozygous *In 3a* strain also carries the y_1 (colorless endosperm) allele in chromosome 6.

The following procedure was used: Plants homozygous for *In 3a* and the a_1 and y_1 alleles were crossed with 15 different inbred lines, R2, R4, Oh45, Oh41, M14, K4, I205, C103, 5120B, K187–2, 38–11, Oh7, WF9, W26, and R59. All of these lines carry the A_1 (colored aleurone) and Y_1 (yellow endosperm) alleles. The F_1 plants, $\frac{In\ 3a\text{-}a\ y}{N\quad A\ Y}$ in constitution, were (1) backcrossed to the inversion parent and (2) self-pollinated. Four genotypic classes of seeds occurred on the backcrossed ears. The chance segregation of Y:y on these ears was utilized to furnish a supplementary linkage test for agronomic characters influenced by genes on chromosome 6. The four classes of seeds were planted in separate rows in 12 replicated plots.

Seeds on the self-pollinated ears were of three types with regard to the tested segment. The kernels of the selfed ears were planted at random without classifying for aleurone or endosperm color in two rows of approximately 145 plants in each row. The F_2 plants were detasselled and intervening rows of an a_1 tester were used as the pollen source. At harvest time, the F_2 plants were classified according to the kernel color on the ears. Comparisons among the different genotypes of the Y locus were not made in the F_2 populations.

In both F_2 and backcross tests, plants of the different genotypes were examined with respect to the following agronomic characters: ear height, maturity, grain yield, kernel row number, and ear number. Kernel weight and number of florets per row were studied only in the backcross progenies. In addition, ovule abortion was determined on a few ears of each genotype. With all characters except grain yield and ear number, only the uppermost ears were examined. Kernel row number was counted in the middle portion of the first ears. Kernel weight was determined by shelling the kernels from the middle part of ears picked at random from the first six replicates of the backcross population of each genotype. Percent of ovule abortion was determined on four ears chosen at random from each of the genotypic classes. Floret number per row was calculated by counting the total florets of an ear and dividing by the row number. Four ears chosen at random from each class were examined.

In general, data obtained from the backcross progenies are presented in a dif-

ferent manner than the information from the F_2 populations. Each replicate of the backcross test consisted of four rows, *Aa Yy*, *Aa yy*, *aa Yy*, and *aa yy* in genotype. When linkage of the agronomic character with chromosome 3 genes is being tested, the data from the *Aa Yy* and *Aa yy* rows are averaged and compared with the average of the *aa Yy* and *aa yy* classes. When linkage with chromosome 6 is studied, the average of the *Aa Yy* and *aa Yy* is compared with the average of the *Aa yy* and *aa yy* classes. The basic values dealt with here are the averages per replication. The data from the F_2 progenies are based on averages of individual plants rather than on rows or replications.

In tests of the significance of differences between genotypes, the "t" value was used. With the backcross data, except the data for floret number per row, the paired method (LEONARD and CLARK 1950) was employed. With the F_2 data and the backcross data for floret number per row, the unpaired method was used.

RESULTS

Characters associated with loci in the long arm of chromosome 3

In the course of this experiment two regions of the maize genome were tested for the presence of loci affecting agronomic traits—a portion of the long arm of chromosome 3 including the A_1 locus and a segment of chromosome 6 in which linkage with the gene Y_1 is detectable. The experimental data relating to these two regions are presented in separate sections. In order to avoid complex terminology, the designations *aa*, *Aa*, and *AA* are used in both text and tables to indicate the class homozygous for inversion *3a*, the class heterozygous for the inversion, and the homozygous normal class, respectively.

Ear height (Table 1): The backcross data for ear height were obtained in 1955 and 1956. In 1955, the comparisons of ear height involved only 13 of the 15 inbred lines. In all comparisons, the *Aa* class consistently had greater ear height than the corresponding *aa* class with the single exception of I205 in the 1956 data. All differences were significant.

The F_2 data for ear height were collected from 14 inbred lines in 1956. The results of the *Aa* vs. *aa* comparisons are generally in agreement with those obtained from the backcross materials. The *Aa* class, except for I205, had greater ear heights than the *aa* class. However, in the F_2 data, the differences in ear height between the two classes were not significant for the inbreds R4, M14, and C103. In the *AA* vs. *aa* comparison, 12 of the 14 inbred lines had a greater ear height in the *AA* than in the *aa* class and significant differences were found in seven of these inbreds. With the remaining two inbreds, the ear height of the *aa* class exceeded that of the *AA*. The difference was significant for I205 but not for M14.

These results indicate that genes affecting ear height are present in the long arm of chromosome 3 of the inbred lines and that their effect is exhibited in the homozygous as well as in the heterozygous condition. In the majority of inbreds, the *AA* and the *Aa* classes are similar in ear height and both classes exceed the *aa* class in this trait. The increase in ear height is due to genes in the A-segment from the inbred line rather than interaction of genes from the inbred and the inversion

TABLE 1

Ear height of plants from crosses of $\frac{\text{In a}}{\text{N A}} \times \frac{\text{In a}}{\text{In a}}$ *(B. C.) and self-pollinations of* $\frac{\text{In a}}{\text{N A}}$ *(F_2)*

	B. C.						F_2					
	1955			1956			1956					
	Av. ear ht. per plant in cms			Av. ear ht. per plant in cms			Av. ear ht. per plant in cms			t value		
	Aa	*aa*	t value	*Aa*	*aa*	t value	*AA*	*Aa*	*aa*	*AA vs. Aa*	*AA vs. aa*	*Aa vs. aa*
R4	------	------	--------	114	106	4.60**	91	94	90	1.52	0.15	1.62
R2	------	------	--------	129	124	6.28**	106	106	96	0.00	3.28**	4.16**
Oh45	115	101	13.15**	115	100	7.91**	94	88	77	2.04*	5.78**	4.61**
Oh41	119	108	9.83**	128	120	5.65**	100	110	100	2.66**	0.00	2.63**
M14	96	92	2.84**	108	103	6.42**	70	76	73	2.95**	1.11	0.87
K4	126	112	7.35**	131	121	6.85**	------	------	------	------	------	------
I205	116	107	4.84**	108	109	0.51	90	105	111	5.15**	6.38**	1.98
C103	108	97	7.68**	122	116	3.67**	84	85	82	0.32	0.53	1.13
5120B	114	101	8.64**	125	114	7.46**	106	111	105	1.68	0.57	2.31**
K187-2	114	93	10.20**	113	93	10.41**	107	104	85	1.42	7.24**	7.36**
38-11	122	111	5.52**	128	120	5.37**	128	122	100	1.87	7.24**	6.31**
Oh7	125	99	13.13**	113	98	12.16**	126	124	109	0.39	4.32**	4.37**
WF9	103	98	2.25*	107	105	2.44*	101	102	96	0.25	1.30	2.15*
W26	104	92	7.64**	120	112	7.07**	100	102	88	0.58	3.62**	5.37**
R59	120	110	9.00**	125	114	10.37**	125	123	108	0.71	4.47**	4.98**

* Significant at 5% level.
** Significant at 1% level.

strain. Thus, there is no evidence of heterotic effects in the chromosome 3 segment when tested for genes controlling ear height.

Maturity (Table 2): Maturity data were obtained from the backcrossed materials in 1955 and 1956, and from the F_2 materials in 1956. The backcross data obtained in the two years are not consistent. Divergent results were also found for backcross and F_2 populations in 1956.

Maturity in corn is markedly affected by environmental factors and the inheritance of this character is known to be complex. The data obtained show some discrepancies which are difficult to explain. Therefore no general conclusions can be made concerning the inheritance of maturity. There is some indication that the inbred line Oh45 has a gene or group of genes for lateness in the long arm of chromosome 3. With this inbred, later maturity of the *Aa* class was observed in backcross populations of both years and later maturity of the *AA* class in the F_2 tests. The inbred WF9 possesses a gene or genes for earliness in the same chromosomal segment when tested against the *In 3a* strain. In this inbred, a trend toward lateness in maturity from *AA* to *Aa* to *aa* classes was observed in both the backcross and F_2 populations.

Grain yield (Tables 3 and 4): Comparisons for grain yield were made using

the *Aa* and *aa* plants of the backcross populations grown in the summers of 1954, 1955, and 1956. Of the 14 inbred lines tested in 1954, significant differences were found for two inbred lines, M14 and K187–2. In both of these, the *Aa* class was superior in grain yield to the *aa* class. Thirteen inbreds, including M14 and K187–2, were tested in 1955. No significant differences in yield between the two classes were found. In 1956, significant differences were observed in five of the 15 inbreds tested, namely R4, Oh41, 38–11, Oh7, and K187–2. In all cases the *Aa* class was superior to the *aa* in grain yield.

The fact that the class homozygous for the inversion never exceeded the heterozygous class in yield is significant. A reduction in yield of the *Aa* class might be expected because of the ovule abortion associated with the heterozygous inversion. The mechanism of "selective orientation" described by STURTEVANT and BEADLE (1936) does not function efficiently in the case of *In 3a* and, as a consequence, the basal megaspore sometimes receives a deficient chromosome and abortion results. The ovule abortion in *In 3a* heterozygotes determined by the author is 15.75 percent. The reduced seed set on ears of the heterozygous class does not lead to a noticeable effect on yield, however, since the loss in number of kernels is largely compensated by the increased kernel weight, as will be shown in a later section. Significant differences in yield in favor of the *Aa* class are therefore meaningful and valid in spite of the presence of ovule abortion.

TABLE 2

Maturity of plants from crosses of $\frac{\text{In a}}{\text{N A}} \times \frac{\text{In a}}{\text{In a}}$ *(B. C.) and self-pollinations of* $\frac{\text{In a}}{\text{N A}}$ *(F_2)*

	B. C.						F_2					
	1955			1956			1956					
	Av. days from planting to half silking			Av. days from planting to half silking			Av. days from planting to silking			t value		
	Aa	*aa*	t value	*Aa*	*aa*	t value	*AA*	*Aa*	*aa*	*AA* vs. *Aa*	*AA* vs. *aa*	*Aa* vs. *aa*
R4	.....	.....	.....	69.2	69.7	2.08	65.1	64.9	64.6	0.54	1.28	0.54
R2	.....	.....	.....	68.8	69.1	1.73	63.7	62.4	63.2	3.52**	1.12	1.98*
Oh45	67.1	65.5	11.00**	66.3	65.2	3.67**	63.4	61.3	61.4	5.38**	3.89**	0.10
Oh41	70.0	69.2	3.04**	69.7	69.4	1.42	65.5	65.6	64.8	0.32	1.68	2.61**
M14	67.7	67.6	0.44	68.4	68.3	0.36	62.3	61.8	62.6	1.41	1.00	2.17*
K4	73.4	72.8	2.10	71.9	71.3	1.87	.....	.....	.....	.....	.....	.....
I205	68.9	68.5	0.85	66.9	66.2	1.85	62.1	62.8	63.0	1.33	1.89	0.68
C103	69.8	70.4	1.80	68.3	68.9	2.22*	65.2	65.0	65.5	0.37	0.47	0.95
5120B	69.5	68.8	2.50*	67.4	67.5	0.50	65.3	65.1	65.9	0.51	1.18	1.87
K187–2	68.2	67.2	2.81**	65.2	64.9	1.43	63.7	63.6	63.0	0.15	1.35	1.32
38–11	69.1	68.3	3.43**	67.0	66.9	0.40	66.5	66.6	66.0	0.12	0.78	1.05
Oh7	69.5	68.6	3.14**	65.3	65.2	0.40	66.4	65.5	65.5	2.18*	1.63	0.00
WF9	65.7	65.8	0.52	62.9	64.1	6.32**	62.4	62.7	64.4	0.72	3.35**	3.41**
W26	67.0	66.3	2.59*	66.1	66.5	1.43	63.9	63.0	63.2	2.05*	1.26	0.50
R59	69.4	69.0	1.68	66.2	66.5	2.64*	72.6	70.2	70.9	4.06**	2.15*	1.08

* Significant at 5% level.
** Significant at 1% level.

The backcross data reveal that some inbred lines possess genes in the long arm of chromosome 3 which give an increase in yield when tested against the same segment of the *In 3a* strain. It is also apparent that certain inbred lines do not show such an effect. This may be due to absence of the specific alleles needed or to their being subliminal in a changed background. Discrepancies in the grain yield data obtained in the three summers may be caused by environmental differences.

A better understanding of the action of the genes for yield can be gained from an examination of the F_2 data. Of the eight inbred lines tested in 1954 and the 15 studied in 1956, less than half showed significant differences in the *Aa* vs. *aa* comparisons. In these cases the *Aa* class was superior in yield to the *aa* class.

The *Aa* and *aa* classes are present in both backcross and F_2 populations. If the heterotic effect on yield were wholly due to the genes located within the chromosomal segment under test, the results of the comparison of *Aa* vs. *aa* in the F_2 and backcross populations should be identical since the same genotypic classes are being studied in each case. This was true for the comparisons of ear height presented in a previous section, but does not hold in the data for yield. For example, in 1956 significant differences in yield between the *Aa* and *aa* classes were found with the inbred lines R4, K187–2, and 38–11 in the backcross populations but not in the F_2 populations. On the other hand, significant differences were found with

TABLE 3

Grain yield and ear number of plants from crosses of $\frac{\text{In a}}{\text{N A}} \times \frac{\text{In a}}{\text{In a}}$ *(B. C.)*

	Grain yield									Ear no.		
	1954			1955			1956			1956		
	Av. wt. per rep. in lbs			Av. wt. per rep. in lbs			Av. wt. per rep. in lbs			No. of ears per rep.		
	Aa	*aa*	t value	*Aa*	*aa*	t value	*Aa*	*aa*	t value	*Aa*	*aa*	t value
R4	3.96	3.69	1.80	.....	.....	.....	3.89	3.58	6.20**	17.7	16.9	1.51
R2	4.06	4.05	0.10	.....	.....	.....	3.37	3.36	0.17	14.2	14.8	2.23*
Oh45	4.37	4.08	1.80	3.54	3.60	0.86	3.35	3.17	2.00	13.1	13.2	0.27
Oh41	4.35	4.40	0.30	3.03	2.96	0.58	3.49	3.24	4.17**	16.9	16.0	1.96
M14	4.44	3.93	2.80**	3.46	3.44	0.20	3.57	3.48	1.50	15.8	16.7	1.99
K4	4.50	4.15	1.60	2.72	2.72	0.00	3.55	3.41	1.52	18.5	18.2	0.48
I205	3.98	3.23	1.30	3.20	3.02	1.50	3.05	3.11	0.90	12.6	13.0	2.19
C103	5.33	5.01	1.30	2.88	2.73	1.55	3.38	3.29	1.32	13.5	13.2	0.72
5120B	3.72	3.75	0.20	2.43	2.56	1.08	3.79	3.58	2.14	15.5	14.3	2.17
K187–2	3.82	3.07	2.90**	2.92	2.97	0.38	2.92	2.65	2.45*	15.1	13.0	4.75**
38–11	4.30	4.32	0.10	2.95	3.09	0.78	3.62	3.39	2.20*	16.0	14.5	2.57*
Oh7	3.63	3.72	0.40	2.75	2.84	0.60	3.56	3.19	3.36**	18.0	14.5	6.44**
WF9	3.90	4.23	1.80	3.23	3.24	0.09	3.36	3.41	1.40	12.5	12.8	1.08
W26	3.68	4.00	1.40	2.73	2.67	0.63	3.29	3.40	1.11	13.5	14.8	2.79*
R59	.....	.....	.....	3.54	3.71	1.13	4.05	3.92	1.55	16.8	17.6	1.20

r of grain yield and ear no. (1956 data) = +0.855 (significant at 1% level).
* Significant at 5% level.
** Significant at 1% level.

TABLE 4

Grain yield and ear number of plants from self-pollinations of $\frac{\text{In a}}{\text{N A}}$ *(F_2)*

	Grain yield												Ear no.					
	1954						1956						1956					
	Av. wt. per plt. in gms			t value			Av. wt. per plt. in gms			t value			Av. ear no. per plt.			t value		
	AA	*Aa*	*aa*	*AA vs. Aa*	*AA vs. aa*	*Aa vs. aa*	*AA*	*Aa*	*aa*	*AA vs. Aa*	*AA vs. aa*	*Aa vs. aa*	*AA*	*Aa*	*aa*	*AA vs. Aa*	*AA vs. aa*	*Aa vs. aa*
R4	102.0	122.9	124.4	2.20*	2.10*	0.20	72.9	96.2	101.8	3.58**	4.60**	1.54	1.03	1.18	1.18	1.93	1.85	0.00
R2	.....	.....	.....	.....	.....	.....	99.2	115.9	108.5	2.42**	1.65	1.48	1.21	1.15	1.09	1.05	1.68	0.99
Oh45	132.8	117.2	113.1	1.40	1.70	0.50	118.2	131.2	108.9	2.52**	1.52	4.82**	1.13	1.18	1.05	0.81	1.52	2.45**
Oh41	.....	.....	.....	.....	.....	.....	110.5	116.4	103.3	1.00	0.99	2.11*	1.20	1.31	1.13	1.22	0.74	1.96
M14	124.9	131.5	103.5	0.80	2.30*	3.40**	106.8	125.8	113.7	4.23**	1.34	2.43**	1.03	1.14	1.13	2.33**	2.07*	0.17
I205	98.2	148.0	153.1	5.60**	4.80**	0.60	94.7	104.2	107.3	1.44	1.59	0.42	1.02	1.08	1.18	1.30	2.48**	1.53
C103	135.2	142.8	126.3	1.00	1.00	2.10*	99.1	122.9	103.7	3.08**	0.47	2.53**	1.04	1.00	1.00	1.79	1.15	0.00
5120B	.....	.....	.....	.....	.....	.....	101.8	117.6	109.5	2.68**	0.99	1.28	1.12	1.14	1.23	0.31	1.33	1.31
K187–2	101.0	127.4	126.2	3.30**	2.60**	0.10	116.0	113.7	103.5	0.38	2.06*	1.96	1.36	1.36	1.07	0.00	3.99**	4.21**
38–11	.....	.....	.....	.....	.....	.....	95.1	111.4	98.1	2.04*	0.36	1.76	1.00	1.16	1.00	2.47**	0.00	2.61**
Oh7	.....	.....	.....	.....	.....	.....	119.0	133.6	113.1	1.98*	0.71	2.74**	1.17	1.25	1.00	0.92	2.25*	2.89**
WF9	121.5	130.4	125.7	1.00	0.50	0.60	112.1	116.8	116.5	0.74	0.59	0.05	1.19	1.10	1.06	1.45	2.03*	0.65
W26	.....	.....	.....	.....	.....	.....	82.3	101.1	98.2	4.29**	3.15**	0.58	1.07	1.19	1.15	2.16*	1.24	0.60
R59	133.8	150.9	124.2	2.10*	1.20	4.20**	92.1	115.3	88.2	3.22**	0.52	3.74**	1.22	1.35	1.19	1.36	0.27	1.67

r of yield and ear no. for *AA vs. Aa* (1956 data) = +0.439 (Not significant).
r of yield and ear no. for *AA vs. aa* (1956 data) = +0.738 (Significant at 1% level).
r of yield and ear no. for *Aa vs. aa* (1956 data) = +0.531 (Significant at 5% level).
* Significant at 5% level.
** Significant at 1% level.

inbreds Oh45, M14, C103, and R59 in the F_2 populations but not in the backcross progenies. The differences in the relation of *Aa* to *aa* in these two populations cannot be attributed wholly to enviornmental modifications.

Although the heterozygosity of both the backcross and F_2 progenies is 50 percent, their chromosomal constitution with respect to the participation of the inbred and inversion parents is different. Moreover, after one generation of selfing there may be a greater loss of genic interaction in the F_2 than in the backcross progenies. Thus, the genic background of the chromosomal segment under test is different in the backcross and F_2 populations. If grain yield in corn is the result of the cooperation of a great number of genes distributed throughout the genome, the difference in genic background of the two populations could influence the total yield to such an extent that a difference in the yield of *Aa* and *aa* classes might not be apparent. The genes for yield in the controlled chromosomal segment no doubt react differently with the altered genic backgrounds.

Most of the inbred lines showed no significant differences in yield of the *AA* and *aa* classes. With a few inbreds, such as R4 and W26, the chromosomal segment being tested was less efficient than the same segment from the *In 3a* strain when studied in homozygous condition against identical genetic backgrounds. In the comparisons of *AA* vs. *Aa* plants, almost all the *Aa* classes had greater yield than the *AA* and significant differences were found for many inbreds.

The data for grain yield indicate that no single factor is responsible for this trait. Significant increases in yield are found when a small segment representing 1/15 of the total genetic make-up of the plant is substituted in a constant genic background and it may be concluded that genes concerned in the expression of yield are present within this segment. However, their effect is certainly modified by the action of genes in the same and in other chromosomes. Environment also plays a part in the determination of yield as is indicated by the year to year variation in the results. In conclusion, it appears that grain yield is affected by specific genes in chromosome 3, by modifying genes in other chromosomes, and by the environment.

Components of yield: In order to learn more about the determination of grain yield, a study was made, similar to that of LENG (1954), of heterotic effects in the components of yield. Data were taken for kernel row number, kernel weight, number of florets per row, and ear number.

Kernel row number (Table 5): The backcross data for row number were collected from 13 inbreds in 1955 and from 15 inbreds in 1956. Significant differences in row number between the *Aa* and *aa* classes were found for inbred lines I205 and K187-2 in both 1955 and 1956; Oh7 and W26 showed significant differences only in 1955. With inbred I205 the *Aa* class had a higher row number than the *aa* while the reverse was true of the other inbred lines. From previous data (LENG, unpublished), I205 is known to have a row number of 20.4 per ear and K187–2 has 13.6 rows per ear. It would appear that some of the genes controlling this difference are located in the long arm of chromosome 3, i.e., line I205

possesses genes for high row number in this segment while K187–2 possesses genes for low row number.

The F_2 data for row number obtained from 14 inbreds in 1956 indicate a decrease in row number from classes *aa* to *Aa* to *AA* in most of the inbreds. These data are generally in agreement with those from the backcross populations with the exception of the entry for I205.

Kernel weight (Table 6): Data for kernel weight from 13 inbreds in 1955 and from 15 inbreds in 1956 were obtained from the backcross populations only. In all comparisons the *Aa* class had greater kernel weight than the *aa* and significant differences were found for most of the inbreds.

The excess in kernel weight of the class heterozygous for the inversion over the homozygous class may be due either to genes from the inbred lines or more likely to the higher ovule abortion on ears of *Aa* plants. In order to determine whether a relation exists between ovule abortion and kernel weight, measurements of ovule abortion on ears of both heterozygous and homozygous classes of 15 inbred lines were made in 1956. In every inbred the *Aa* class had greater ovule abortion than the *aa*. On the average the ovule abortion of the *Aa* ears was 15.75 percent (11.3–

TABLE 5

Kernel row number of plants from crosses of $\frac{\text{In a}}{\text{N A}} \times \frac{\text{In a}}{\text{In a}}$ *(B. C.) and self-pollinations of* $\frac{\text{In a}}{\text{N A}}$ *(F_2)*

	B. C.						F_2					
	1955			1956			1956					
	Av. row no. per ear			Av. row no. per ear			Av. row no. per ear			t value		
	Aa	*aa*	t value	*Aa*	*aa*	t value	*AA*	*Aa*	*aa*	*AA* vs. *Aa*	*AA* vs. *aa*	*Aa* vs. *aa*
R4	.....	.....	.....	15.25	15.43	1.20	15.33	16.00	16.35	1.61	2.09*	0.82
R2	.....	.....	.....	13.80	13.88	0.73	14.80	15.40	15.20	2.07*	1.15	0.06
Oh45	13.54	13.50	0.31	13.55	13.60	0.50	14.62	14.56	14.35	0.22	0.30	0.76
Oh41	13.19	13.23	0.17	12.98	13.03	0.31	13.31	13.44	14.07	0.45	1.76	2.02*
M14	13.69	13.63	0.43	14.77	15.06	2.18	15.91	15.84	16.23	0.27	0.88	1.39
K4	13.13	13.36	1.35	13.63	13.63	0.00	.....	.....	.....	.....	.....	.....
I205	13.83	13.33	2.27*	14.22	13.79	4.30**	15.00	15.21	15.69	0.57	1.75	1.28
C103	12.02	12.06	0.29	12.29	12.59	2.00	12.44	12.88	13.22	1.47	1.85	1.14
5120B	13.73	13.96	1.64	13.48	13.59	1.66	14.52	15.35	15.68	2.39**	3.27**	1.03
K187–2	14.08	14.76	3.58**	13.66	14.18	3.06**	14.68	15.03	15.93	1.25	3.75**	3.93**
38–11	14.68	14.67	0.06	13.63	13.79	1.33	16.38	16.52	16.71	0.32	0.75	0.44
Oh7	13.43	14.04	6.70**	12.88	13.22	1.32	13.95	14.73	16.00	2.13*	4.92**	2.90**
WF9	14.38	14.28	0.69	14.73	14.88	0.94	14.83	15.47	16.52	1.98*	4.56**	3.15**
W26	13.61	14.09	3.20**	14.30	14.50	1.43	14.37	14.91	14.84	2.07*	1.40	0.25
R59	13.49	13.69	2.00	12.75	12.86	0.58	13.00	13.62	13.92	1.55	2.34**	0.72

* Significant at 5% level.
** Significant at 1% level.

TABLE 6

Kernel weight and ovule abortion of plants from crosses of $\frac{\text{In a}}{\text{N A}} \times \frac{\text{In a}}{\text{In a}}$ *(B. C.)*

	Kernel weight						Ovule abortion		
	1955			1956			1956		
	Av. wt. of 1,000 kernels in gms			Av. wt. of 1,000 kernels in gms			Ovule abortion per ear in %		
	Aa	*aa*	t value	*Aa*	*aa*	t value	*Aa*	*aa*	*Aa-aa*
R4	.....	.....	.....	187	166	2.94*	15.2	2.9	12.3
R2	.....	.....	.....	192	181	2.57*	12.8	2.0	10.8
Oh45	270	241	2.37	231	201	3.17*	18.7	3.7	15.0
Oh41	239	212	4.95**	199	173	3.93**	14.3	3.9	10.4
M14	237	205	7.23**	189	173	4.07**	15.6	3.2	12.4
K4	248	206	10.87**	178	159	2.28	17.9	5.8	12.1
I205	254	222	3.76**	197	184	1.86	15.2	2.6	12.6
C103	270	263	1.70	221	205	2.48	14.8	3.9	10.9
5120B	252	220	5.30**	235	207	8.80**	15.3	2.4	12.9
K187-2	244	211	2.74*	202	177	4.41**	20.9	2.8	18.1
38-11	248	217	2.83*	218	188	6.06**	17.4	3.2	14.2
Oh7	233	211	4.94**	214	205	1.59	11.3	2.7	8.6
WF9	241	202	7.21**	223	186	12.41**	19.2	2.6	16.6
W26	236	209	3.69**	220	197	3.71**	11.8	4.4	7.4
R59	256	217	4.61**	246	228	1.62	15.9	3.5	12.4

r of kernel weight and ovule abortion (1956 data) = +0.603 (Significant at 1% level).
* Significant at 5% level.
** Significant at 1% level.

19.2 percent) while that of the *aa* ears was 3.31 percent (2.0–5.8 percent). The difference was 12.45 percent (7.4–18.1 percent).

The coefficient of correlation between the percentage of excess kernel weight of the *Aa* class over the *aa* ($\frac{Aa-aa}{aa}$) and the excess ovule abortion of the *Aa* class over the *aa* (*Aa–aa*) was determined using inbred lines as entities and based on the backcross data of 1956. This value was approximately +0.603, which is highly significant at the one percent level. The correlation coefficient indicates a positive correlation between kernel weight and ovule abortion. The excess kernel weight of the *Aa* class is due mainly to higher ovule abortion and the consequent decrease in spatial barriers of kernel growth. However, the effect of genes from the inbred lines cannot be entirely ruled out.

Floret number per row: Since ears of the *Aa* class were not completely filled due to the ovule abortion associated with the heterozygous inversion, an analysis of floret number per row, instead of kernel number, was made in 1956 using the backcross material. A higher floret number per row of the *Aa* over the *aa* class was found for eight of the 15 inbred lines but a significant difference was observed only with R59. With the remaining seven inbred lines, the *aa* class had a higher floret number than the *Aa* but all differences were small and not statistically significant. In general there appears to be no precise relation between floret

number and yield. The same backcross data in which a significantly higher floret number of *Aa* over *aa* was observed for R59 showed no significant increase in yield of *Aa* over *aa* plants for this inbred line.

Ear number (Tables 3 and 4): In the backcross populations of 1956 significant differences in ear number between the *Aa* and *aa* classes were found in five of 15 inbred lines. With inbreds K187–2, 38–11, and Oh7, the *Aa* class exceeded the *aa* while with inbreds R2 and W26 the *aa* class had the higher ear number.

Close examination of the backcross data for grain yield and for ear number of the same year reveals that increases in grain yield are often related to increases in ear number (Table 3). Significant superiority in yield of the *Aa* class over the *aa* was found with five inbred lines, R4, Oh41, K187–2, 38–11, and Oh7, in 1956. With all five inbred lines the *Aa* class also had a higher ear number than the *aa* and significant differences were observed in inbreds K187–2, 38–11, and Oh7. The value of the correlation coefficient was +0.855, which is significant at the one percent level.

The F_2 data for ear number obtained in 1956 show similar relationships between ear number and grain yield (Table 4). The correlation coefficients obtained were +0.439 (not significant), +0.738 (significant at the 1% level), and +0.531 (significant at the 5% level) for *AA* vs. *Aa*, *AA* vs. *aa*, and *Aa* vs. *aa*, respectively.

The following conclusions were reached from the study of the components of grain yield: (1) The heterotic effect on yield observed with some inbreds in the class heterozygous for the inversion is not related to kernel row number or floret number per row. (2) The relationship between the increased yield of the heterozygous class and its excess kernel weight is obscure because of the presence of ovule abortion. (3) There is a strong correlation between yield and ear number. The increase in ear number might be ascribed to ovule abortion, but this explanation is rendered unlikely by SPRAGUE's finding that plants heterozygous for the pericentric inversion 5*a*, which has a considerable amount of ovule abortion, but did not differ in ear number from homozygous sibs. Thus, the differences in grain yield of the *AA*, *Aa*, and *aa* classes may be attributed mainly to genes for ear number located in the tested segment of chromosome 3.

Association of quantitative characters with the Y_1 locus

Data collected from the *Aa Yy*, *Aa yy*, *aa Yy* and *aa yy* classes in the study of quantitative characters associated with the *A* locus were also used to test linkage between genes affecting agronomic traits and the gene *Y* on chromosome 6. As no inversion is involved in chromosome 6, crossing over between *Y* and linked loci would occur with normal frequency and this would tend to reduce the differences found between the *Yy* and *yy* classes for certain agronomic characters.

Ear height (Table 7): The data for ear height analyzed with respect to the *Yy* and *yy* classes were obtained from 13 inbreds in 1955 and from 15 inbreds in 1956. The *yy* plants had a greater ear height than the *Yy* in all comparisons except two. Significant differences were found with four of the 13 inbred lines tested in 1955 and with ten of 15 involved in 1956. On the whole, the data, especially that

TABLE 7

Ear height of plants from crosses of $\frac{Y}{y} \times \frac{y}{y}$

	1955			1956		
	Av. ear ht. per plt. in cms			Av. ear ht. per plt. in cms		
	Yy	*yy*	t value	*Yy*	*yy*	t value
R4	------	------	------	106	113	8.46**
R2	------	------	------	123	129	4.75**
Oh45	107	110	2.20*	107	109	0.82
Oh41	113	114	0.18	122	125	2.15
M14	92	96	3.13**	104	107	2.10
K4	110	110	0.00	125	127	1.32
I205	110	118	4.34**	106	111	3.43**
C103	102	104	1.07	115	121	6.33**
5120B	107	108	0.25	116	123	5.48**
K187–2	102	105	1.75	103	103	0.00
38–11	119	115	2.16	123	125	3.85**
Oh7	110	114	2.21*	102	108	3.20**
WF9	100	104	1.62	102	109	5.41**
W26	98	97	0.72	114	118	5.15**
R59	115	115	0.00	117	121	3.30**

* Significant at 5% level.
** Significant at 1% level.

of 1956, indicate that a gene or group of genes for ear height is linked with the *Y* locus on chromosome 6. The more effective genes are associated with the *y* allele and the less effective genes with the *Y* allele. Since linkage between the *Y* locus and the ear height genes is dissipated by crossing over, the size of the differences between *Yy* and *yy* classes may not be truly representative of the capacities of the genes in this chromosome.

Maturity (Table 8): The *yy* class was later in maturity than the *Yy* in all comparisons. Significant differences were found for two of the 13 inbreds in 1955 and for nine of the 15 inbreds in 1956. The consistent trend toward late maturity of the *yy* class found with every inbred in both years as well as the fact that many of the inbreds tested in 1956 showed significant differences in the two classes, leads to the conclusion that a gene or genes for maturity is associated with the *Y* locus—the allele for earliness linked with the *Y* allele and the allele for lateness with the *y* allele. The discrepancies in the maturity data obtained in 1955 and 1956 may be attributed to differences in the two growing seasons.

Grain yield (Table 9): Data for grain yield were obtained from 13 inbreds in 1954 and 1955, and 15 inbreds in 1956. Significant differences in yield between the *Yy* and *yy* classes were found only with inbreds K4 and C103 in 1955 and with Oh45 and 5120B in 1956, although a greater yield of the *Yy* class over the *yy* was observed in most inbreds in 1954 and 1956. With inbred K4, the *yy* plants had a greater yield than the *Yy* while in the other three inbred lines the *Yy* plants were superior in yield to the *yy*.

TABLE 8

Maturity of plants from crosses of $\frac{Y}{y} \times \frac{y}{y}$

	1955			1956		
	Av. days from planting to half silking			Av. days from planting to half silking		
	Yy	*yy*	t value	*Yy*	*yy*	t value
R4	.....	.....	.....	68.7	70.2	7.89**
R2	.....	.....	.....	68.5	69.4	3.31**
Oh45	66.1	65.5	1.48	65.2	66.3	4.35**
Oh41	69.3	69.8	1.48	69.4	69.7	1.65
M14	67.5	67.7	0.77	68.0	68.7	1.96
K4	72.5	73.5	12.50**	71.0	72.2	4.91**
I205	69.3	69.4	0.37	66.3	66.8	0.90
C103	69.9	70.3	1.12	67.9	69.3	4.92**
5120B	68.7	69.5	2.68**	67.4	67.6	1.00
K187–2	67.4	68.0	1.85	65.0	65.1	0.63
38–11	68.8	69.1	2.92	66.8	67.1	1.32
Oh7	68.8	69.3	1.81	65.0	65.5	2.50*
WF9	65.6	65.9	1.24	63.2	63.8	3.73**
W26	66.6	67.1	1.19	65.6	67.0	7.80**
R59	69.0	69.5	2.08	66.0	66.7	3.09**

* Significant at 5% level.
** Significant at 1% level.

TABLE 9

Grain yield of plants from crosses of $\frac{Y}{y} \times \frac{y}{y}$

	1954			1955			1956		
	Av. wt. per rep. in lbs.			Av. wt. per rep. in lbs.			Av. wt. per rep. in lbs.		
	Yy	*yy*	t value	*Yy*	*yy*	t value	*Yy*	*yy*	t value
R4	3.88	3.77	0.66	.....	.....	.....	3.80	3.68	1.86
R2	4.16	3.95	1.57	.....	.....	.....	3.44	3.29	1.69
Oh45	4.19	4.25	0.39	3.51	3.63	0.71	3.38	3.15	3.29**
Oh41	4.37	4.38	0.05	3.02	2.96	0.55	3.40	3.34	0.71
M14	4.26	4.11	0.72	3.45	3.45	0.00	3.58	3.46	0.90
K4	4.19	4.46	1.22	2.45	2.85	3.00**	3.50	3.45	0.67
I205	3.63	3.58	0.10	3.13	3.19	0.38	3.12	3.04	0.82
C103	.....	.....	.....	2.90	2.66	3.00**	3.37	3.26	1.37
5120B	3.83	3.63	1.20	2.54	2.40	1.17	3.80	3.57	2.30*
K187–2	3.56	3.33	0.84	3.02	2.87	1.50	2.82	2.75	0.96
38–11	4.42	4.20	1.64	3.14	2.87	1.23	3.45	3.54	1.00
Oh7	3.76	3.59	0.81	2.74	2.85	0.92	3.38	3.37	0.15
WF9	4.15	3.98	0.75	3.29	3.29	0.00	3.34	3.44	1.43
W26	3.98	3.70	1.18	2.75	2.51	2.00	3.37	3.32	0.58
R59	.....	.....	.....	3.70	3.54	1.33	4.05	3.92	1.62

* Significant at 5% level.
** Significant at 1% level.

In the majority of the inbred lines, there was no significant difference in yield between the two classes. Moreover, inconsistent results from year to year were found in the data. In general, the data do not reveal any differences between the inbreds and the inversion strain with regard to genes for yield associated with the *Y* locus on chromosome 6.

Components of yield. Kernel row number (Table 10): In almost all of the comparisons the *Yy* class had a greater number of rows than the *yy*. Significant differences were found for six of the 13 inbreds tested in 1955 and for seven of the 15 inbreds tested in 1956. The row number of the *yy* class exceeded that of the *Yy* in two cases, K4 in 1955 and R4 in 1956, but the differences were very small and not statistically significant. The data indicate that a gene or group of genes for row number is linked with the *Y* locus. The *y* allele is associated with lower kernel row number and the *Y* allele with higher row number.

Kernel weight: Data for kernel weight were obtained from 13 inbreds in 1955 and from 15 inbreds in 1956. Although a significant difference in kernel weight between the *Yy* and *yy* plants was found in one inbred, Oh45, in 1956, in most inbred lines there was no great difference between the two classes. Thus, if genes affecting kernel weight are associated with the *Y* locus in the inbred stocks, they are similar to those present in the inversion stock.

Floret number: The data on floret number were obtained from fifteen inbred lines in 1956. A significant difference in floret number per row between the *Yy*

TABLE 10

Kernel row number of plants from crosses of $\frac{Y}{y} \times \frac{y}{y}$

	1955			1956		
	Av. row no. per ear			Av. row no. per ear		
	Yy	*yy*	t value	*Yy*	*yy*	t value
R4	.....	.....	.....	15.30	15.39	0.78
R2	.....	.....	.....	14.18	13.52	5.19**
Oh45	13.55	13.51	0.22	13.74	13.39	3.04**
Oh41	13.48	12.96	4.33**	13.18	12.83	2.45**
M14	14.00	13.33	4.79**	15.11	14.72	2.05
K4	13.24	13.29	0.28	13.83	13.44	3.15**
I205	13.78	13.46	1.28	14.01	14.01	0.00
C103	12.07	12.02	0.38	12.54	12.36	1.38
5120B	14.04	13.66	2.92**	13.77	13.31	3.89**
K187–2	14.65	14.21	3.67**	14.08	13.73	2.67**
38–11	14.65	14.53	0.57	13.82	13.62	1.47
Oh7	13.85	13.63	2.44*	13.09	12.99	0.58
WF9	14.38	13.80	3.41**	15.01	14.62	3.45**
W26	14.04	13.84	1.05	14.49	14.31	1.04
R59	13.76	13.46	1.88	12.92	12.68	1.77

* Significant at 5% level.
** Significant at 1% level.

and *yy* classes was observed in WF9 only. There is no evidence in the other inbred lines of a genetic difference with regard to floret number.

Ear number (Table 11): With 12 of the 15 inbred lines tested in 1956, the *yy* plants had a higher ear number than the *Yy*. Significant differences were found for five inbred lines, R2, K4, K187–2, Oh7, and W26. With two inbreds, 38–11 and R59, the *Yy* class was superior in ear number over the *yy* but the differences were small and not statistically significant. The data reveal that a gene or genes associated with ear number may be present on chromosome 6—the gene for high ear number linked with the *y* allele and that for low ear number with the *Y* allele.

The results described above for the components of yield are somewhat puzzling when compared with the data on grain yield. The yield data show few significant differences between the *Yy* and *yy* classes, but the data for kernel row number and ear number indicate the presence on chromosome 6 of genes affecting certain components of yield. The same set of backcross data was studied in all cases except that the data on floret number and ear number were based only on 1956 tests. The backcross data for grain yield of 1956 showed superiority of the *Yy* class for the lines Oh45 and 5120B. Contributing to the greater yield of *Yy* plants in Oh45 tests were the greater kernel weight and higher row number found for that inbred. The excess yield of *Yy* plants in 5120B tests was at least partly due to the significantly higher number of rows found with that inbred. The significant differences between *Yy* and *yy* classes in the data on ear number and row number which do not affect the over-all yield data may be accounted for since in most cases the increase in ear number favors the *yy* class while the increase in row number favors the *Yy*

TABLE 11

Ear number of plants from crosses of $\frac{Y}{y} \times \frac{y}{y}$

	Av. ear no. per rep.		
	Yy	*yy*	t value
R4	17.2	17.4	0.54
R2	14.0	15.0	2.20*
Oh45	13.0	13.4	1.20
Oh41	16.4	16.5	0.21
M14	16.0	16.5	0.70
K4	17.2	19.5	4.40**
I205	12.5	13.1	1.50
C103	13.0	13.7	1.54
5120B	14.9	14.9	0.00
K187–2	13.5	14.7	4.48**
38–11	15.4	15.2	0.35
Oh7	15.1	17.4	5.65**
WF9	12.5	12.8	1.65
W26	13.8	14.6	2.96**
R59	17.6	16.9	0.92

* Significant at 5% level.
** Significant at 1% level.

class. It would seem that the opposing influences of these two components are balanced so that no effect on over-all yield is exhibited.

DISCUSSION

The method of DOBZHANSKY and RHOADES (1938) in which paracentric inversions are utilized as a means of locating desirable genes in maize has been shown to be effective in the present study as well as in that of SPRAGUE. SINGLETON (1941) questioned the value of this method since he believed that the differences between plants of *Aa* and *aa* genotypes would be masked by the heterozygous constitution of genes on the other nine chromosomes. He suggested that the F_1 plants be backcrossed for many generations to the inbred parent, retaining the inversion in heterozygous state. It would then be possible to derive two stocks of identical background but differing with regard to the inverted segment. These could be crossed to an inbred line and the resulting F_1's compared for differences in agronomic characters.

There would seem to be no necessity for such an elaborate series of crosses if measurable and significant differences occur between plants derived from the backcross to the inversion strain. SPRAGUE (1941), using as F_1 the cross of an *In 5a* strain and a 15-generation dent inbred, found significant differences in plot yield and kernel weight between backcross classes heterozygous and homozygous for the inversion. In the present experiment significant differences both in yield and in other agronomic characters were observed in a number of cases. It is true that the expression of such differences may depend on a favorable environment since significant differences occurring in one summer were not exhibited the next year. It is also possible that for certain inbreds, even under the most favorable conditions, the action of the genes in the chromosome 3 segment may be eclipsed by the overriding influence of the residual genotype. However, utilization of the modified method of SINGLETON would not eliminate this difficulty since the hybrids in which the comparisons are to be made would be heterozygous for many genes which could largely determine the character under consideration.

There is no doubt that the method of DOBZHANSKY and RHOADES is more effective than the use of single marker genes in determining the location of favorable genes. When backcross data for ear height obtained by these two methods are compared, it is obvious that more consistent and greater differences are found in the *Aa* vs. *aa* tests (Table 1) than in the *Yy* vs. *yy* tests (Table 7) in the same two year period. Although the more significant results from the *Aa* vs. *aa* tests is attributed in part to the presence of major genes for ear height on chromosome 3 and less effective ones on chromosome 6, it is also true that, in the *Yy* vs. *yy* tests, linkage of *Y* and the genes under study is not complete and the distinction between the two classes is therefore lessened by crossovers.

The tests for ear height reveal that there are genes associated with this trait in the long arm of chromosome 3 and in chromosome 6 near the *Y* locus. The more efficient genes for ear height are carried by the chromosome 3 segment of the inbred lines and by the chromosome 6 derived from the inversion strain. Thus, each

parent contributes a different set of favorable genes. Considering only those loci on chromosome 3, the F_2 data (Table 1) rule out interaction in the heterozygote *Aa* of genes from the inbred and the inversion strain since an increase in ear height is exhibited in plants with the homozygous *AA* condition as well as in the *Aa* types. No complete F_2 data are available to test the interaction among genes for ear height on chromosome 6 but the superiority of *yy* over *Yy* plants in itself negates any marked interaction between parental loci in this region. Thus, genes in the long arm of chromosome 3 and on chromosome 6 near the *Y* locus make independent contributions to ear height.

The additive effect of genes for ear height is evident from an inspection of the data in Table 12 which lists the mean ear height of the *Aa Yy*, *Aa yy*, *aa Yy* and *aa yy* classes in the 1955 and 1956 growing seasons. Since the chromosome 3 segment from the inbred lines carried more potent genes for ear height than did the segment associated with the *y* allele of the inversion strain, it might be expected that the *aa Yy* class would have the lowest placed ears, with progressively higher placed ears on the *aa yy*, *Aa Yy* and *Aa yy* plants, respectively. When the class values for the inbreds tested in both 1955 and 1956 are averaged and arranged in ascending order, the genotypic classes assume the above mentioned rank. In general, the differences in ear height between any two classes can be predicted if the average contribution of the genes in chromosome 3 and the genes in chromosome 6 is known.

TABLE 12

Ear height of plants from crosses of $\frac{\text{In a}}{\text{N A}}\frac{\text{Y}}{\text{y}} \times \frac{\text{In a}}{\text{In a}}\frac{\text{y}}{\text{y}}$

	1955				1956			
	Av. ear height per plt. in cms				Av. ear height per plt. in cms			
	Aa Yy	*Aa yy*	*aa Yy*	*aa yy*	*Aa Yy*	*Aa yy*	*aa Yy*	*aa yy*
R4	------	------	------	------	110	117	103	107
R2	------	------	------	------	127	130	120	128
Oh45	114	117	99	103	115	115	98	101
Oh41	119	118	107	109	126	130	119	121
M14	94	99	91	94	108	108	101	105
K4	127	127	113	113	130	132	121	122
I205	115	123	105	113	105	111	107	111
C103	106	111	97	97	119	124	112	119
5120B	112	116	103	99	122	127	110	118
K187-2	113	115	90	95	113	113	93	93
38-11	125	122	113	108	126	129	120	119
Oh7	123	127	100	97	112	115	95	101
WF9	102	108	97	102	104	110	101	108
W26	105	104	92	90	117	123	110	113
R59	119	121	111	108	123	127	111	116
Average	113.4	116.0	101.4	102.2	117.1	120.7	108.1	112.2

F for classes (1955 data) = 44.95 (Significant at 1% level).
F for classes (1956 data) = 39.30 (Significant at 1% level).

ROBINSON, COMSTOCK and HARVEY (1949) made an extensive investigation of the ratio of dominance to additive genetic variance in open-pollinated varieties of corn as well as in crosses of inbreds. They found no evidence of overdominance in ear height. The present study confirms their results; the equality of *AA* and *Aa* classes in the F_2 tests and the superiority of *yy* individuals over *Yy* in the backcross rule out a serious consideration of the overdominance hypothesis.

With a number of inbred lines in our experiment, a significantly greater yield was found in the class heterozygous for the inversion than in either of the homozygotes. The fact that comparisons involving the same inbred line may show significance in the F_2 data and not in the backcross has been interpreted as an indication of epistatic action of background genes. The difference between F_2 and backcross data is certainly not conclusive evidence of genic interaction and more extensive comparisons showing consistent differences in the same direction would be desirable. However, since genes associated with components of yield have been reported on chromosomes other than 3 and were found in this study on chromosome 6, the conclusion is inescapable that loci on many different chromosomes participate in the determination of yield and would be expected to influence the expression of genes on chromosome 3.

With regard to the components of yield, it is interesting to note that their effects are sometimes complementary and sometimes antagonistic. In one set of data, the *Yy* plants, on the whole, possessed more rows per ear but fewer ears per plant than the *yy* individuals. As a result of these opposing tendencies, no significant differences in over-all yield between the two classes were observed. In this case a study of the components of yield reveals the presence of genes which would otherwise have been undetected.

The component most responsible for the greater yield of the inversion heterozygotes in this study is the number of ears per plant. The correlation coefficients for the yield and ear number data in comparisons of *Aa* vs. *aa* were significant at the one percent level in backcross tests (Table 3) and at the five percent level in the F_2 tests (Table 4). LENG (1954) observed that kernel number per row was the component showing the most consistent superiority in the F_1 hybrid. LENG's study involved the entire genome whereas only a small segment was tested by the author. One might conclude that the major genes for yield in the chromosome 3 segment affect ear number, while genes for kernel number per row play a dominant part when the whole genome is considered.

Among the various components of yield, row number has been a favorite subject of genetic investigations over a number of years. EMERSON and EAST (1913) showed in considerable detail that the inheritance of row number could be interpreted in Mendelian terms. LINDSTROM (1931) found linkage between row number genes and the genes for cob and pericarp color (*P*), aleurone color (*R*), and endosperm color (*Y*). EMERSON and SMITH (1950), in a study of the inheritance of row number in crosses among inbred lines, found that it is possible to accumulate genes for high row number by selection. MANN (1950), by the use of translocations, found that genes in chromosome 3 near the ts_4 locus, in chromo-

some 6 near the *Y* locus, and on certain other chromosomes all influence the number of rows per ear.

The location of genes for row number in the long arm of chromosome 3 and on chromosome 6 near the *Y* locus by means of the DOBZHANSKY and RHOADES method in the present study confirms the findings of LINDSTROM and of MANN. The genes producing a high number of rows are associated in most cases with the chromosome 3 segment of the inversion strain and with the *Y* allele of the inbred lines. A dosage effect is indicated for the genes in chromosome 3 since a decrease in row number from the *aa* to *Aa* to *AA* classes was observed with most of the inbreds. When the *A* and *Y* linkage groups are followed simultaneously, the combined effects of the two systems are revealed. Among the four backcross classes (Table 13), the highest row number occurs in *aa Yy* plants and the lowest in *Aa yy*, while the *Aa Yy* and *aa yy* classes are intermediate. This ranking reflects the summation of the separate contributions of the segments involved in each genotype. Although the differences between classes are not spectacular, an additive type of action is indicated for the genes in chromosomes 3 and 6 determining row number. This finding confirms the conclusion of EMERSON and SMITH (1950) that it is possible to accumulate genes for high row number by selection.

SUMMARY

In this study the method of DOBZHANSKY and RHOADES for locating favorable genes in maize by the use of paracentric inversions was adopted. A stock contain-

TABLE 13

Kernel row number of plants from crosses of $\frac{\text{In a}}{\text{N A}}\frac{\text{Y}}{\text{y}} \times \frac{\text{In a}}{\text{In a}}\frac{\text{y}}{\text{y}}$

	1955				1956			
	Av. row no. per ear				Av. row no. per ear			
	Aa Yy	*Aa yy*	*aa Yy*	*aa yy*	*Aa Yy*	*Aa yy*	*aa Yy*	*aa yy*
R4	.....	.....	.....	.....	15.3	15.1	15.2	15.6
R2	.....	.....	.....	.....	14.1	13.5	14.2	13.5
Oh45	13.7	13.4	13.4	13.6	13.6	13.4	13.8	13.3
Oh41	13.4	13.0	13.5	12.9	13.0	12.9	13.3	12.7
M14	14.2	13.2	13.8	13.4	14.9	14.6	15.3	14.8
K4	13.0	13.2	13.4	13.3	13.9	13.3	13.7	13.6
I205	14.0	13.5	13.5	13.4	14.2	14.3	13.8	13.7
C103	12.1	11.9	12.0	12.1	12.5	12.1	12.6	12.6
5120B	13.9	13.5	14.1	13.8	13.8	13.2	13.7	13.4
K187–2	14.3	13.8	14.9	14.6	13.8	13.5	14.4	13.9
38–11	14.3	14.4	14.9	14.6	13.6	13.6	14.0	13.6
Oh7	13.5	13.4	14.2	13.8	12.9	12.8	13.2	13.1
WF9	14.5	13.7	14.2	13.9	15.0	14.4	14.9	14.8
W26	13.8	13.6	14.3	14.0	14.5	14.1	14.5	14.5
R59	13.7	13.3	13.8	13.6	12.8	12.7	13.0	12.7
Average	13.7	13.4	13.9	13.6	13.9	13.6	14.0	13.7

F for classes (1955 data) $=8.08$ (Significant at 1% level).
F for classes (1956 data) $=9.83$ (Significant at 1% level).

ing a paracentric inversion in chromosome 3, which effectively suppressed crossing over in one half the length of the long arm, was crossed with each of 15 inbred lines. Comparisons of agronomic characters were made within the F_2 and backcross populations in classes heterozygous and homozygous for structural differences. Since the average residual genotype is constant in all classes within a population, significant differences between classes can be attributed to the effects of the segment under study. A region on chromosome 6 could be followed by means of the Y_1 locus but it is not transmitted as a unit because of the occurrence of crossing over. Measurements were made on ear height, maturity, yield, row number, kernel weight, floret number and ear number. All conclusions made with regard to the presence of genes having heterotic effects are necessarily restricted to a particular genic and environmental background.

From these experiments, the following conclusions may be reached: Genes increasing ear height are present in chromosome 6 of the *In 3a* strain and in the long arm of chromosome 3 in most of the inbred lines studied. These genes do not show overdominance. Genes for earliness are present in the long arm of chromosome 3 in the inbred WF9 and in the chromosome 6 region of many other inbred lines. Certain inbred lines possess genes in the long arm of chromosome 3 which give an increase in yield. However, the expression of these genes varies with the genic background and environmental conditions. Thus, heterosis for yield is due to genes within the inverted segment, modifying genes on other chromosomes, and the interaction with enviornmental factors. The *In 3a* segment contains genes for high row number while the majority of the inbred lines possess such genes on chromosome 6. A striking correlation was observed between yield and ear number. Genes for high ear number are located on chromosome 3 of many inbred lines and on chromosome 6 from the inversion strain. Ovule abortion in the class heterozygous for the inversion does not seriously affect the data on yield since the decrease in number of kernels was largely compensated by excess kernel weight. The method of DOBZHANSKY and RHOADES can be used successfully to locate genes for ear height, row number, and ear number. It is less effective when applied to data on yield and maturity where interactions with the residual genome and the environment play an important part.

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EXPERIMENTS ON THE EFFECTS OF HOMOZYGOSITY AND HETEROZYGOSITY ON THE RATE OF DEVELOPMENT IN DROSOPHILA MELANOGASTER

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IN 1956 the authors made preparations for and started experiments on the effect of selection pressures of different strengths on irradiated populations of *Drosophila melanogaster*. When planning these experiments several problems on the relative viabilities of homozygotes and heterozygotes presented themselves. One of these concerned comparisons of homozygotes and different heterozygotes in multiple allelic loci.

In the selection pressure experiments, the strength of the pressure was regulated by letting a given number of larvae compete for a standard amount of food: a large number in high selection pressure populations but a small number in low selection pressure populations (BONNIER, JONSSON and RAMEL 1958). When many larvae compete for a limited amount of food the variation in the time which it takes for the larvae to reach the adult stage is large. It seems obvious that, at least as a rule, those individuals who reach eclosion early are more viable than those who need a long time for their development. In other words, among the characters which may be used for defining and measuring viability and selective ability, rate of development may be taken as one. We decided, therefore, to use a technique for studying rate of development similar to that we already had worked out in our irradiation experiments.

At the end of 1956, preparations were made to produce suitable strains for the experiments and the experiments themselves were performed in 1957. However, with the experience gained and after studying the results it was found necessary to make further experiments. New preparations were, therefore, made in 1957 and a new set of experiments were performed in 1958.

Experiments of the 1957 series

Synthesis of strains: Five alleles of the white locus were used, *viz.* +, w^{co}, w^{a}, w^{t}, w. The wild type chromosome was taken from flies in our stock bottle labelled w^{aat}. The stock of this bottle had been propagated for many years without any check of its content. As we were unable to distinguish the eye color of this stock from that of wild type flies, and as it proved to be dominant over all of the w alleles tested, we symbolize it here by the sign +. In chart I the synthesis is shown diagrammatically.

Experimental technique: All experiments were performed as larval competition experiments. After 16 hours of oviposition (from 5 p.m. to 9 a.m. of the next

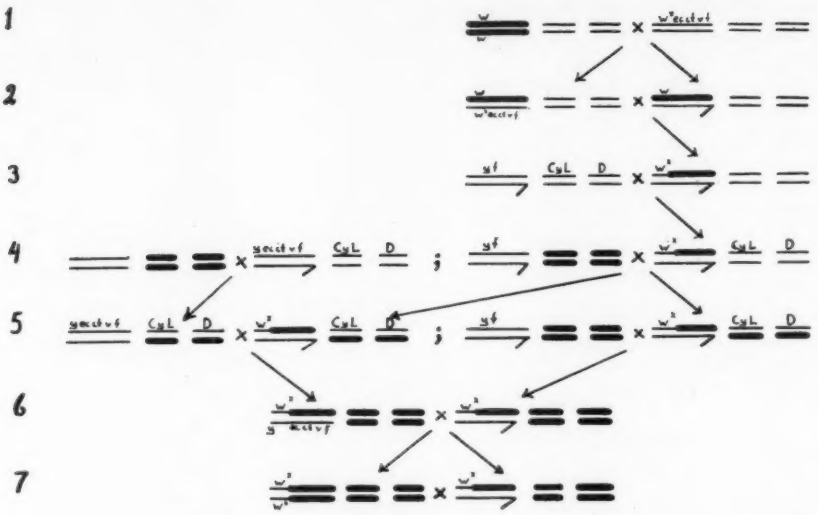


CHART I.—Synthesis of strains used in the 1957 series of experiments. Isolated chromosomes, with regard to which the strains were rendered isogenic, are drawn in heavy lines. Females were made homozygous for one *w* containing X chromosome as indicated in generation 1. Other females were made homozygous for a set of the two long autosomes containing wild type genes as indicated in generation 4. The chart shows how the gene *w* was substituted by other *w* alleles (collectively named w^x), and how these were combined with the set of isogenous autosomes. Apart from *w* (locus 1.5) and its alleles, the following sex-linked marker genes were used: *ec* (5.5), *ct* (20.0), *v* (33.0), *f* (56.7). The symbol *yf* refers to double attached XXY females homozygous for *y* and *f*. *M5*, *CyL*, and *D* are symbols for crossing over inhibiting dominant markers.

day) and a further 28 hours of incubation. freshly hatched larvae were collected and transferred to vials with food of standard type. The amount of food was in every vial about 12 ml, though no exact weighings or measurements were made. The number of larvae transferred to each separate vial was always 200—which means a strong competition between the larvae. Of these, 100 were taken from one of the prepared strains or cross between two of them, and 100 from another of these strains or cross between two other of them. All vials were incubated at 25°C in an incubator, not used for other purposes when these experiments took place.

Experiment 1957:1: Though the primary purpose for setting up the present series of experiments was to compare heterozygotes with homozygotes or with other kinds of heterozygotes it seemed appropriate to start with a larval competition experiment between the pure strains. As mentioned above we had produced five homozygous coisogenic strains. Ten different kinds of comparisons of two by two were, hence, possible to make. One of these, *viz.* that between w^t and *w*, could not be fulfilled because of the uncertainty in many individual cases in distinguish-

ing between these two eye colors. The nine remaining comparisons were, however, performed. Females were allowed to oviposit on four consecutive days (as mentioned above for 16 hours each time) and from each of these one vial with freshly hatched larvae was started for each of the nine comparisons. Table 1

TABLE 1

Experiment 1957:1

Results from competitions between pure strains. Figures show totals from four vials for each kind of comparison. Each vial started with 200 larvae of which 100 were taken from each of the two strains shown in the left margin of the table

Larvae from strains	Adults emerged up to 9 days 18 hours after beginning of mothers' ovipositions				Total number of adults emerged			
	Females		Males		Females		Males	
+ and w^{co}	74 +,	42 w^{co}	87 +,	29 w^{co}	161 +,	177 w^{co}	163 +,	149 w^{co}
+ and w^a	94 +,	12 w^a	67 +,	12 w^a	190 +,	181 w^a	160 +,	168 w^a
+ and w^t	85 +,	19 w^t	46 +,	14 w^t	202 +,	180 w^t	157 +,	136 w^t
+ and w	81 +,	31 w	95 +,	29 w	170 +,	161 w	189 +,	151 w
w^{co} and w^a	66 w^{co},	20 w^a	60 w^{co},	28 w^a	164 w^{co},	182 w^a	149 w^{co},	173 w^a
w^{co} and w^t	45 w^{co},	20 w^t	33 w^{co},	19 w^t	183 w^{co},	177 w^t	153 w^{co},	144 w^t
w^{co} and w	40 w^{co},	30 w	35 w^{co},	18 w	166 w^{co},	170 w	151 w^{co},	143 w
w^a and w^t	50 w^a,	17 w^t	34 w^a,	14 w^t	174 w^a,	180 w^t	168 w^a,	135 w^t
w^a and w	56 w^a,	37 w	51 w^a,	22 w	190 w^a,	183 w	165 w^a,	142 w

shows the results. The total number of observed adults was 5,987 or 83 percent of the 7,200 larvae. As an equal number (100) of larvae of each of the two competing types was collected one should expect that the adults should come out in a 1:1 proportion. As may be seen, the observed proportions after ceasing of eclosion are generally in good agreement with the expectations; among the 18 proportions for females and for males there were only two cases for which the probability of deviation was less than 0.10. From this point of view one would, therefore, be right in saying that the two competing types in all cases were of practically equal viability.

The vials with the emerging flies were emptied on several occasions before the eclosion ceased. The proportions between the two competing types were, at the early counts, very far from 1:1. Table 1 gives also the sums for the four vials with regard to the flies which had reached eclosion within nine days and 18 hours after the beginning of their mothers' ovipositions. When w^{co}, w^a, w^t or w competed with wild type, much more than 50 percent were wild type; when w^a, w^t or w competed with w^{co} much more than 50 percent were w^{co}; and when w^t or w competed with w^a much more than 50 percent were w^a. That the wild type larvae in the present material developed faster than any of the other types is thus obvious. Concerning the w^{co} larvae the absolute numbers of the adults were practically identical whether the larvae had to compete with +, w^t or w. The seriation of the percentages of w^{co} when competing with + (31%), w^a (72%), w^t (67%) or w (61%) must, therefore, be taken with some caution. The w^a larvae, on the other

hand, show the same kind of seriation whether one judges from the absolute figures or from the percentages. The total number of w^a in competition with +, w^{co}, w^t and w was respectively 24 (13%), 48 (28%), 84 (73%), 107 (64%). From this case one may conclude that the rate of development of larvae of Drosophila is determined, not only by the strength of selection pressure and by their own genotypes, but also by the genotypes of those other larvae with which they have to compete.

One finds the following seriation of competition ability: $+ > w^{co} > w^a > w^t$ and w. It seems, therefore, as if this seriation follows the degree of deviation from normality, in this case expressed as the deviation in eye color of the adults from the wild type red eye color. (Compare, however, experiment 1958:5, Table 9, Figures 13, 14).

Experiment 1957:3: From the five different genotypes, +, w^{co}, w^a, w^t and w one may get ten different heterozygotes, and these may be combined two by two in 45 different ways. Several of the genotypically different heterozygotes are phenotypically indistinguishable and, therefore, only 10 of the 45 comparisons were performed (Experiment 1957:2). As the results proved to be of no great interest, their description is omitted here.

In the hope of getting more information we decided, however, to make one more experiment, this time by always letting two kinds of wild type female larvae compete. However, to proceed in this way it is necessary to identify the genotypes of the observed females by progeny testing them individually. The experiment, as performed, included collection of 12,800 larvae, from which 5,428 females emerged; of these 4,818 or 88.8 percent could be identified by progeny tests.

Heterozygous females may be produced in two different ways, either by crossing wild type homozygous females with nonwild type males, or by making the reciprocal cross. The types of male larvae with which the female larvae have to compete depend thus upon the way in which the crosses are made. For instance, the production of $+/w^{co}$ and $+/w^a$ female larvae for a competition experiment may be made so as to have them compete with only + males, with + and w^{co} males, with + and w^a males, or with w^{co} and w^a males. To evaluate the competition abilities of the female larvae from these four types of crosses proved to be more difficult than we had expected. We confine, therefore, the present description to those four crosses in which the competition was between homozygotes +/+ and that kind of heterozygotes which themselves were produced by crossing wild type homozygous females +/+ to nonwild type males (Table 2). For each comparison one vial was raised on each of two consecutive days, and, as before, each vial contained 100 larvae from each of two crosses. The table shows the numbers of observed females and the genotypes and numbers of the identified ones. The percentages refer in this table to the number of observed females which could be identified by progeny tests.

Because of the fact that eclosion this time began somewhat later than in experiment 1957:1 we have, for the demonstration in the table, chosen ten days and 18 hours after beginning of oviposition as a suitable early stage for the comparisons.

TABLE 2

Experiment 1957:3

Results from competitions between wild type homozygous and heterozygous females, when the crosses were made in such a way that all males were wild type. The genotypes of the females were identified by individual progeny tests. Under the sign "%" the numbers of observed females which were possible to identify in this way are given as percents of the total number of females observed at the corresponding stage. Figures show totals from two vials for each kind of competition. Each vial started with 200 larvae of which 100 were taken from each of the two crosses shown in the table. The sign "w^x" means any of w^{co}, w^a, w^t, w

	Larvae from crosses shown below																			
	+/+ × + and +/+ × w^{co}				+/+ × + and +/+ × w^a				+/+ × + and +/+ × w^t				+/+ × + and +/+ × w				Total			
		Identified				Identified				Identified				Identified				Identified		
	Observed	+/+	+/w^{co}	%	Observed	+/+	+/w^a	%	Observed	+/+	+/w^t	%	Observed	+/+	+/w	%	Observed	+/+	+/w^x	%
10 days 18 hours after beginning of mothers' oviposition	35	24	10	97	34	20	8	82	71	37	26	89	21	18	2	95	161	99	46	90
Total eclosion	160	75	75	94	149	70	61	88	181	78	78	86	176	84	77	91	666	307	291	90

TABLE 3

Experiment 1958:1

Total number of adults observed. Figures show totals from four vials per cross. Each vial started with 200 larvae. Autosomal homozygotes are a1/a1; *autosomal heterozygotes are from crosses* a1/a1 ♀♀ × a2/a2 ♂♂

	Larvae from X chromosome crosses shown below									
	+/+ × +		+/+ × w^{co}		+/+ × + w^a		+/+ × w			
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂		
Autosomal homozygotes	414	331	363	339	381	342	422	320		
Autosomal heterozygotes	472	284	447	313	435	342	443	308		

The results are of special interest as they show that in the present experiment the homozygous wild type females on an average reached eclosion earlier than the heterozygous ones; i.e. the homozygotes had a higher competing ability than the heterozygotes.

Experiments of the 1958 series

Experiment 1957:3 had indicated that homozygous wild type female larvae had a higher competing ability than heterozygous wild type female larvae. These results were, however, based on very little material and we decided, therefore, to take up this question for a more thorough investigation.

With the experiences gained from the 1957 series, we found several precautions desirable. These included *inter alia* (1) that all comparisons of competing wild type female larvae should be based on crosses producing males of only one kind, namely, wild type; (2) that temperature influences, as well as other environmental intervial influences, should be more thoroughly levelled out; and (3) that a new and still more careful procedure should be undertaken to make the strains coisogenic with regard to their X chromosomes.

Synthesis of strains: The strains were made coisogenic with regard to the long autosomes (chromosomes 2 and 3), in the conventional way, but this time we produced two different strains each of which was homozygous for chromosomes 2 and 3. These two strains will here be named *a1* and *a2*. The procedure of rendering the strains coisogenic with regard to their X chromosomes started now by isolating one single X chromosome from a wild type stock "Algeria" instead of, as in the 1957 series, from a stock of white. To simplify, we decided to drop w^t from our comparisons, which, therefore, came to include only four w alleles, *viz.* $+$, w^{ra}, w^a, w. The synthesis is shown diagrammatically in chart II.

It should be mentioned that the simultaneous use of several eye color genes made it necessary to test the males of the different generations by crossing them to females homozygous for appropriate eye color genes. All such tests were performed. We had thus produced four X chromosomes containing respectively one of the four white alleles, $+$, w^{ra}, w^a or w and which, at least with regard to the euchromatic region, were identical except for a segment ranging from a point between the loci of *pn* and w to a point between the loci of w and *ec*, i.e. for a segment, the maximal length of which is 4.7 map units. Each of these four X chromosomes were then combined, in one strain, with the autosomal set *a1*, and in a second strain, with the autosomal set *a2*. In all we had thus eight strains for use in the experiments.

Though we did not pay any special regard to the Y chromosome in the procedure of rendering the strains coisogenic, the Y chromosomes of all our final strains happened to have their origin from Y chromosomes in our stock of *pn*. This does not necessarily mean that the different strains got identical Y chromosomes, only that their origin was in a common stock which for many generations was mass propagated.

To chromosome 4 no regard was paid. The final strains might, therefore, have

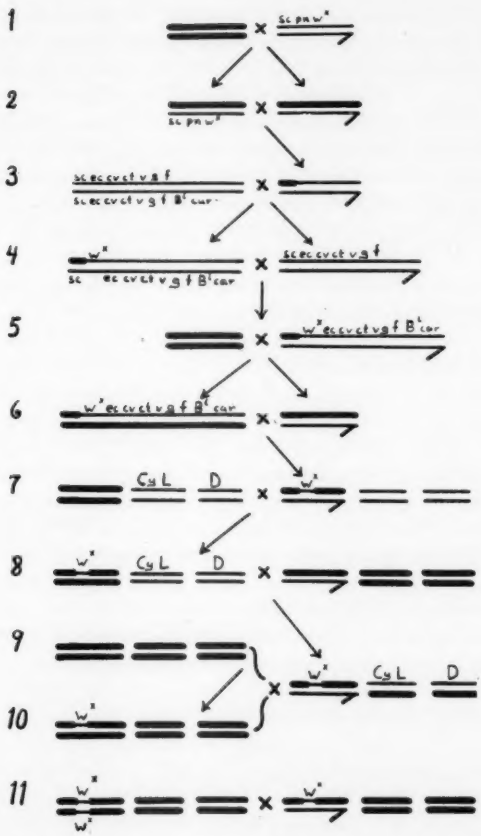


CHART II.—Synthesis of strains used in the 1958 series of experiments. Isolated chromosomes, with regard to which the strains were rendered isogenic, are drawn in heavy lines. The first six generations show the sex chromosomes only. Females were made homozygous for one wild type X chromosome (originating from a stock from Algeria) as indicated in generation 1. Other females were made homozygous for either the one or the other of two different sets of the long autosomes (containing wild type genes), as indicated in generation 8. These two sets are in the text named *a1* and *a2* respectively. The chart shows how the different *w* alleles (collectively named w^r) were combined with one of the autosomal sets. In this way each of the X chromosome were, in different strains, combined with either the set *a1* or the set *a2*. Apart from the sex-linked marker genes mentioned in the text to chart I the following were also used: *sc* (0.0), *pn* (0.8), *cv* (13.5), *g* (44.4), B^i (57.0), *car* (62.5).

been homozygous or heterozygous for this chromosome; and they might also, *inter se*, have been coisogenic or not coisogenic.

Experimental technique: Not only from our experiences gained during the 1957 series of the present experiments but also from earlier studies (BONNIER and JONSSON 1957) we knew that larval growth rate is very sensitive even to small changes in temperature. As this rate possibly also is dependent on other environmental conditions we found it desirable to perform those experiments, the results of which were to be compared, simultaneously. But even with arrangements for a good temperature distribution, it is very difficult to get the variations between different vials to be due only to random causes. In the main experiments of the 1958 series, experiments 1958:1 and 1958:2, we proceeded, therefore, in the following way.

One of the ordinary rooms of the laboratory was equipped with thermo regulators for 25°C and fans circulated the air as evenly as possible. The whole of the development from oviposition to eclosion took place in this room. As will be described below, experiment 1958:1 included comparisons of larvae with four different X chromosomes and two different sets of autosomes. For each of the four X chromosomes, there were raised four cultures in vials containing food and freshly hatched larvae. These vials were put into two trays—one for each of the two autosomal compositions—and arranged within the trays in the way of a 4×4 latin square. The latin squares of the two trays were not identical but drawn at random from those described by FISHER and YATES (1949). Experiment 1958:2 included six X chromosomes, and two autosomal compositions. For each of the six X chromosomes there were raised six cultures in vials which were put into two trays—one for each of the two autosomal compositions—and arranged within the trays in the way of two 6×6 latin squares. It should be stressed that these arrangements in latin squares were not made with the purpose of partitioning the variances in the way such arrangements make possible. The reason was just to try to level out non random intervial environmental differences as much as possible.

After 16 hours of oviposition and a subsequent incubation in 25°C for 28 hours, freshly hatched larvae were transferred to the food in vials arranged in latin squares as just described where they remained undisturbed. From the beginning of eclosion the vials were emptied with four hour intervals, when the adults were counted. The earliest four hour period within which adults had emerged was that which ended 8 days, 14 hours after the beginning of the mothers' ovipositions. The vials were emptied and inspected with regard to eclosion in such four hour periods up to that which ended 12 days, 18 hours after beginning of oviposition. Thereafter the eclosions were checked at longer time intervals; only very few adults appeared in these late periods.

Symbols, diagrams and statistical treatments of the data: The symbols $+$, w^{co}, w^{a} and w refer to X chromosome constitutions and the symbols *a1* and *a2* to the autosomal sets. The maternal chromosomes are in the symbols placed on the left side, and the paternal chromosomes on the right side of the line. The symbol $+/w^{co}$

means, thus, a female the mother of which was wild type and the father of which was w^{co}; and the symbol *a1/a2* refers to flies which have inherited the autosomal set *a1* from their mother and the autosomal set *a2* from their father.

All diagrams are drawn on equal scales. The horizontal scale shows the numbers of days and hours which had elapsed from the beginning of the mothers' ovipositions to the emergence of the adults observed. The vertical scale shows averages referring either to the numbers of directly counted females or males, or —as in most cases all females were phenotypically wild type and hence indistinguishable *inter se*—to the number of females which could be identified with regard to their genotypes by progeny tests. The curves show the average number of adults which had emerged from the beginning of eclosion and up to the moment marked by the corresponding vertical lines (meridians); the points relating to two consecutive such moments are in the diagrams connected by straight lines.

In most of the diagrams a retardation in the rate of eclosion may be seen, e.g., in Figure 1 between 10 days, 2 hours and 10 days, 10 hours. This is due to the rhythm of eclosion: the emergence from the puparia is usually low during the early part of the night.

Comparisons of growth rates shown in Figure 17 are based on an analysis of variance of the usual kind. In all other cases the comparisons are based on t tests. in which the numbers of observed flies are used as variates and in which the number of variates is expressed by the number of vials. In noncompetition experiments the compared differences refer to differences between averages of variates from different vials; in competition experiments, the compared differences are differences of the numbers of the two competing types within the same vial.

The significance level, P, of the differences observed are, for each instance of observation, shown in the bottom of the diagrams by number of asterisks.

no asterisk	$P > 0.05$
one asterisk	$0.05 > P > 0.01$
two asterisks	$0.01 > P > 0.001$
three asterisks	$P < 0.001$

When the diagrams show the results for the two sexes separately the significance asterisks are also given separately for the two sexes or explained in the text to the figures.

A word of caution should be said with regard to biological evaluations and conclusions based on these tests. Competition ability and growth rate is a quantitative character, but when it is measured by *numbers* of flies, one is using a variate which can attain integral values only, i.e. a discontinuous variate. If the two types compared occur in different numbers, their difference cannot be smaller than one, and it is evident that such a difference has a relatively higher influence if the number of flies, or the difference between their numbers, is small than if it is large. This may, when the number mentioned is small, result in too small a difference between the averages compared; but it may also result in too small a sum of squares leading to too large a value of t with the consequence that the significance of a difference may be unduly overestimated. One should, therefore,

remember that in our tests (shown by asterisks in the figures) similar significance levels have, so to say, higher weights when the corresponding number of adults, or difference between their numbers, is large than when it is small.

Main experiments of the 1958 series

Experiment 1958:1: This experiment was a noncompetition experiment. With regard to the X chromosome four types were compared, *viz.* larvae from the following crosses $+/+ \times +$, $+/+ \times w^{co}$, $+/+ \times w^{a}$, $+/+ \times w$. Two hundred freshly hatched larvae from each of these crosses were transferred to each of four vials. All of the crosses were, with regard to the autosomes, made in two different ways: either, all parents were homozygous *a1/a1*, thus producing offspring, the whole of which also was homozygous *a1/a1;* or mothers were *a1/a1* and fathers *a2/a2*, thus producing offspring the whole of which was heterozygous *a1/a2*.

The final results after ceasing of eclosion are given in Table 3. Figures 1A, 1B, 1C show the averages—in the case of autosomal homozygosity *a1/a1*—of the numbers of adult females, which up to the times specified on the horizontal scale had reached eclosion. The solid lines are, in these three figures, identical, and refer to females which were homozygous $+/+$ in their X chromosomes. Figures 2A, 2B, 2C show the corresponding averages in the case of autosomal heterozygosity *a1/a2*.

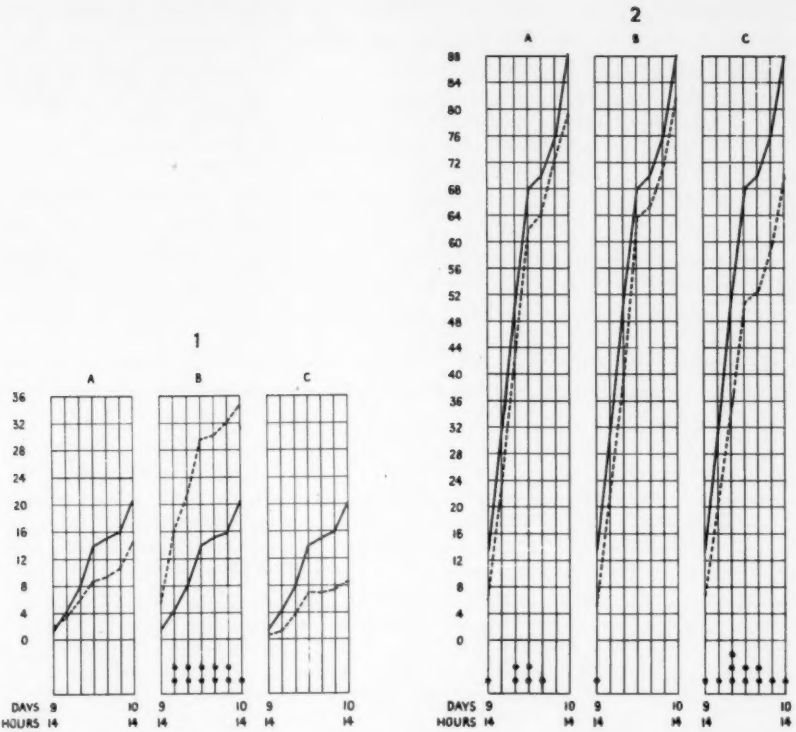
Experiment 1958:2: This experiment was a larval competition experiment. One hundred freshly hatched larvae from each of two crosses were transferred to a common vial. Six combinations of two by two crosses were made, which, with regard to the X chromosomes, were:

$+/+ \times +$ and $+/+ \times w^{co}$
$+/+ \times +$ and $+/+ \times w^{a}$
$+/+ \times +$ and $+/+ \times w$
$+/+ \times w^{co}$ and $+/+ \times w^{a}$
$+/+ \times w^{co}$ and $+/+ \times w$
$+/+ \times w^{a}$ and $+/+ \times w$

The total numbers of adults observed are given in Table 4.

For each of these six combinations six vials were started, and, as in experiment 1958:1, made in two different ways, corresponding to the two autosomal combinations. In one of these all parents of the crosses were autosomal homozygotes *a1/a1*, thus producing larvae which likewise were autosomal homozygotes *a1/a1*. In the second set all mothers were again autosomal homozygotes *a1/a1* but all fathers autosomal homozygotes *a2/a2*, thus producing larvae which were autosomal heterozygotes *a1/a2*.

As all mothers of the crosses in the present experiment were, with regard to the X chromosome. homozygous wild type. $+/+$, all offspring were also wild type. After eclosion the females were, in order to identify their X chromosome genotypes, individually progeny tested in vials, which were incubated at 25°C. If a female was a homozygote, $+/+$, she could produce only wild type sons but if she was a heterozygote, for instance $+/w^{co}$, she should produce two kinds of sons,



FIGURES 1 and 2.—Results from the noncompetition experiment 1958:1 with regard to early part of eclosion of females which were autosomal homozygotes *a1/a1* (Figure 1) and autosomal heterozygotes *a1/a2* (Figure 2). The solid lines are identical in A, B, and C and show eclosions of X chromosome homozygotes +/+. The broken lines show X chromosome heterozygotes: A, $+/w^{co}$; B, $+/w^{a}$; C, $+/w$. Each curve shows averages of four repeats. See also description in text.

wild type sons and w^{co} sons. In those comparisons where the purpose of the progeny test was to identify a female as being homozygous or as being heterozygous, the rule was followed that if, after 14 days of incubation, her male progeny consisted of only wild type males, she was classified as homozygous +/+, provided that the number of males was at least ten. In the few cases in which a female got only wild type sons and these were less than ten, she was excluded from those females which were recorded as "identified". This induces a small bias in favour of heterozygotes; a female producing, say six wild type sons and two w^{co} sons, could with certainty be identified as being a heterozygote $+/w^{co}$; whereas a female producing only eight sons all wild type was nonidentified. Such cases formed, however, a very small group and the bias is certainly of no importance.

TABLE 4

Experiment 1958:2

Total number of adults observed. Figures show totals from six vials per cross. Each vial started with 200 larvae of which 100 were taken from each of the two crosses shown in the table. Autosomal homozygotes are a1/a1; *autosomal heterozygotes are from crosses* a1/a1 ♀♀ × a2/a2 ♂♂. *All flies phenotypically wild type (except six nondisjunctional males not included in the table)*

	Larvae from X chromosome crosses shown below											
	+/+ × + and +/+ × $w^{e\circ}$		+/+ × + and +/+ × w^{a}		+/+ × + and +/+ × w		+/+ × $w^{e\circ}$ and +/+ × w^{a}		+/+ × $w^{e\circ}$ and +/+ × w		+/+ × w^{a} and +/+ × w	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
Autosomal homozygotes	581	573	548	547	600	487	553	522	555	541	522	544
Autosomal heterozygotes	676	467	691	458	668	471	624	524	649	498	672	470

TABLE 5

Experiment 1958:2

Identification of genotypes through progeny tests of individual females. The numbers of females which could be identified are in the table given as percents of the numbers of females actually observed. The stage, 10 days 14 hours from beginning of the mothers' ovipositions, correspond to the end stage of the curves in the diagrams, Figures 3 and 4

	Larvae from X chromosome crosses shown below						
	+/+ × + and +/+ × $w^{e\circ}$	+/+ × + and +/+ × w^{a}	+/+ × + and +/+ × w	+/+ × $w^{e\circ}$ and +/+ × w^{a}	+/+ × $w^{e\circ}$ and +/+ × w	+/+ × w^{a} and +/+ × w	Total no. of females identified
Autosomal homozygotes: Up to 10 days, 14 hrs. from beginning of mothers' ovipositions	95	91	87	96	95	91	826
Total eclosion	72	78	70	75	67	77	2,457
Autosomal heterozygotes: Up to 10 days, 14 hrs. from beginning of mothers' ovipositions	90	96	94	97	95	95	2,525
Total eclosion	80	93	81	84	80	82	3,256

A rather large part of those females which reached eclosion within the period between 10 days, 18 hours and 11 days, 6 hours after beginning of oviposition died without any progeny for the progeny tests. This unfortunate fact, the reason of which is unknown, make comparisons rather worthless between the final numbers of the two competing types. Such comparisons are, however, still possible to make for females reaching eclosion not later than 10 days and 14 hours after beginning of oviposition, i.e. during periods which are of the greatest interest for the present

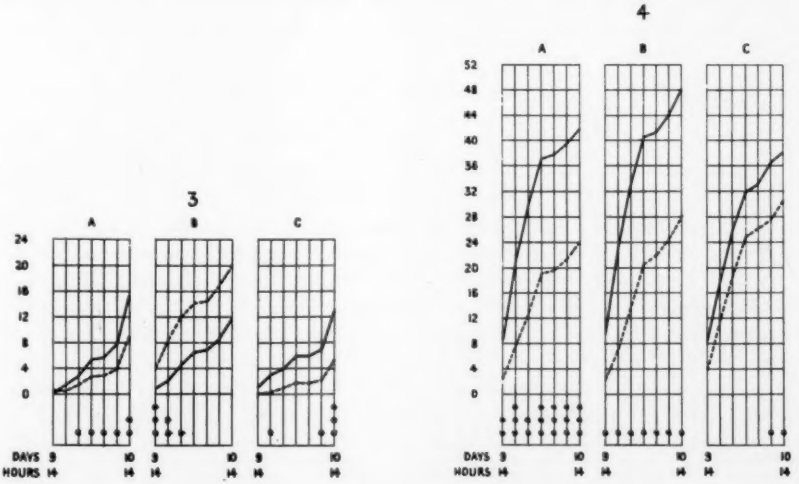
study. Table 4 shows the final results and Table 5 shows the percentages of females which could be identified through progeny tests. The numbers of (identified) females observed during the early part of eclosion are shown in the diagrams Figures 3, 4, 5, 6.

Additional experiments of the 1958 series

As may be seen from the diagrams Figures 1, 3, 5 there was in combination with autosomal homozygosity *a1/a1* a peculiar effect in descendants of w^a males. This "w^a effect", which was quite unforeseen, will be discussed later. It seemed, however, desirable to make some additional experiments to shed, if possible, more light on the causes underlying this effect.

In the 1957 series we had found a seriation of the rates of development of the pure strains *viz.* $+>w^{co}>w^a>w$. It was, hence, also of interest to try to find out if this seriation would hold with the strains used in the 1958 series, especially with regard to the w^a effect. It was, finally, of interest to repeat a part of experiment 1958:2, in the hope that we might identify through progeny tests that part of the wild type females which emerged as adults during the later stages of eclosion.

Experimental technique: Because of other works in our laboratory, these additional experiments could not be performed with all the precautions taken in the 1958:1 and 1958:2 experiments. The vials, standing in trays, were put into a

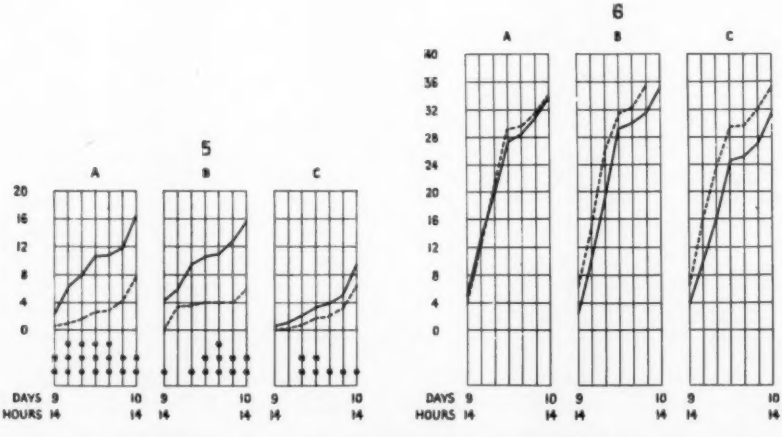


FIGURES 3 and 4.—Results from the competition experiment 1958:2, with regard to early part of eclosion of progeny tested females which were autosomal homozygotes *a1/a1* (Figure 3) and autosomal heterozygotes *a1/a2* (Figure 4). Concerning the X chromosomes the curves show: A, solid line $+/+$, broken line $+/w^{co}$; B, solid line $+/+$, broken line $+/w^a$, C, solid line $+/+$, broken line $+/w$. Each curve shows averages of six repeats. See also description in text.

small incubator at 25°C, and this incubator was not used for other simultaneous purposes. All of the experiments were made with three vials which were put rather randomly into the trays. The vials were emptied and the adults counted on a limited number of occasions. These experiments could not all be made simultaneously.

Experiment 1958:3: This was a competition experiment between pure X chromosome strains of autosomal homozygotes *a1/a1* as well as of autosomal heterozygotes *a1/a2*. The final results are given in Table 7 and the early part of the eclosions is shown on Figures 9 and 10.

Experiment 1958:4: This experiment was a competition experiment in which the females had to be identified through progeny tests as to their genotypes. It included three comparisons *viz.* between $+/+$ and $+/w^{co}$, $+/+$ and $+/w^{a}$, $+/+$ and $+/w$ and was made with autosomal homozygotes *a1/a1* as well as with autosomal heterozygotes *a1/a2*. It included also one comparison between $+/+$ and $+/w^{a}$, in which the autosomal homozygotes were *a2/a2* and the autosomal heterozygotes were *a2/a1*. As is shown in Table 6, a large proportion of the observed females could be identified from the whole of eclosion. The proportions between the total number of the two competing types are also in a good agreement with the expected 1:1 proportions. The probability of deviation, P, is in no case less than 0.2. As the purpose of this experiment was just to test the final proportions between competing types among identified females, only very few counts were made before eclosion had ceased. No counts were made between 9 days, 18 hours



FIGURES 5 and 6.—Results from the competition experiment 1958:2 with regard to early part of eclosion of progeny tested females which were autosomal homozygotes *a1/a1* (Figure 5), and autosomal heterozygotes *a1/a2* (Figure 6). Concerning the X chromosomes the curves show: A, solid line $+/w^{a}$, broken line $+/w^{co}$; B, solid line $+/w^{a}$, broken line $+/w$; C, solid line $+/w^{co}$, broken line $+/w$. Each curve shows averages of six repeats. See also description in text.

TABLE 6

Experiment 1958:4

Identification through progeny tests of individual females. Each vial started with 200 larvae of which 100 were taken from each of the two X chromosome crosses shown in the table. In all crosses except the rightmost one, autosomal homozygotes are a1/a1 *and autosomal heterozygotes from crosses* a1/a1 ♀♀ × a2/a2 ♂♂. *In the rightmost cross autosomal homozygotes are* a2/a2 *and autosomal heterozygotes from crosses* a2/a2 ♀♀ × a1/a1 ♂♂. *Figures show totals from three vials per cross.*

		Larvae from X chromosome crosses shown below							
		+/+ × + and +/+ × w^{ce} +/+		+/+ × + and +/+ × w^a +/+		+/+ × + and +/+ × w +/+		+/+ × + and +/+ × w^a +/+	
			+/w^{ce}		+/w^a		+/w		+/w^a
Autosomal homozygotes	Total identified females	143	139	114	105	116	117	160	143
	Numbers of identified females as percents of actually observed females:								
	10 days, 18 hours after beginning of mothers' ovipositions	98		99		97		98	
	end of eclosion	97		91		93		98	
Autosomal heterozygotes	Total identified females	153	150	145	139	159	162	141	124
	Numbers of identified females as percents of actually observed females:								
	10 days, 18 hours after beginning of mothers' ovipositions	95		98		99		98	
	end of eclosion	94		96		98		97	

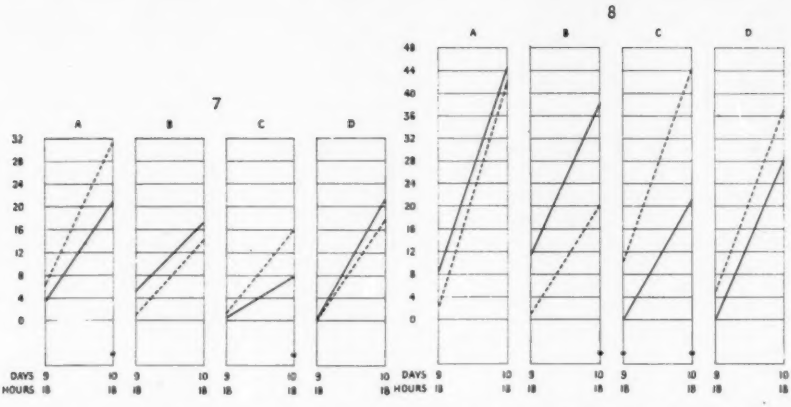
and 10 days, 18 hours after beginning of the mothers' ovipositions; each of the corresponding diagrams, Figures 7 and 8, has thus only two points connected by straight lines.

Experiment 1958:5: This experiment was a noncompetition experiment including X chromosome wild type homozygotes and different wild type heterozygotes. Autosomal homozygotes and heterozygotes were, in this case, *a2/a2* and *a2/a1* respectively. The results are given in Table 9 and Figures 13 and 14.

Experiment 1958:6: This was a competition experiment between pure X chromosome strains. The autosomal homozygotes were *a2/a2* and the autosomal heterozygotes *a2/a1*. The results are given in Table 8 and Figures 11 and 12.

DISCUSSION

The w^a *effect:* From experiments on larval competition between pure strains of the 1957 series (experiment 1957:1, Table 1) it could be shown, that the absolute growth rate of a certain strain depends, apart from environmental conditions, not



FIGURES 7 and 8.—Results from the competition experiment 1958:4 with regard to early part of eclosion of progeny tested females which were autosomal homozygotes *a1/a1* in A, B, and C, and autosomal homozygotes *a2/a2* in D (Figure 7) and autosomal heterozygotes *a1/a2* in A, B, C and autosomal heterozygotes *a2/a1* in D (Figure 8); Concerning the X chromosomes the lines show: A, solid line +/+, broken line $+/w^{co}$; B, solid line +/+, broken line $+/w^{a}$; C, solid line +/+, broken line $+/w$; D, solid line +/+, broken line $+/w^{a}$. Each curve shows averages of three repeats. See also description in text.

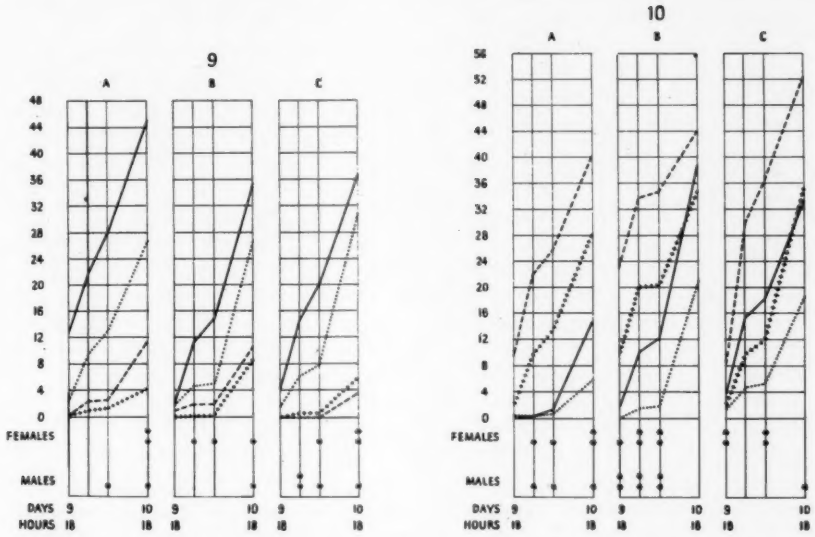
TABLE 7

Experiment 1958:3

Total number of adults observed. Figures show totals from three vials per competition. Each vial started with 200 larvae of which 100 were taken from each of the two strains shown in the table. Autosomal homozygotes are a1/a1, *autosomal heterozygotes are from crosses* a1/a1 ♀♀ × a2/a2 ♂♂

	Larvae from X chromosome strains shown below											
	w^a and +		+		w^a and w^{co}		w^{co}		w^a and w		w	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
Autosomal homozygotes	159	125	128	108	141	145	141	133	148	141	139	130
Autosomal heterozygotes	160	125	154	125	163	124	140	114	115	90	166	115

only on the genotype of the strain itself, but also on the genotypes of those individuals with which the individuals of the strain have to compete. A similar situation occurs also in other organisms, e.g. barley (HAGBERG, personal communication). From the point of view of larval competition ability, the 1957 strains showed a seriation which seemed to follow the degree of deviation of the eye color of the adults from wild type eye color $+ > w^{co} > w^{a} > w^{t} > w$. The strains of the 1958 series did not show this seriation, but this was to a certain extent due to what we have named the "w^{a} effect".



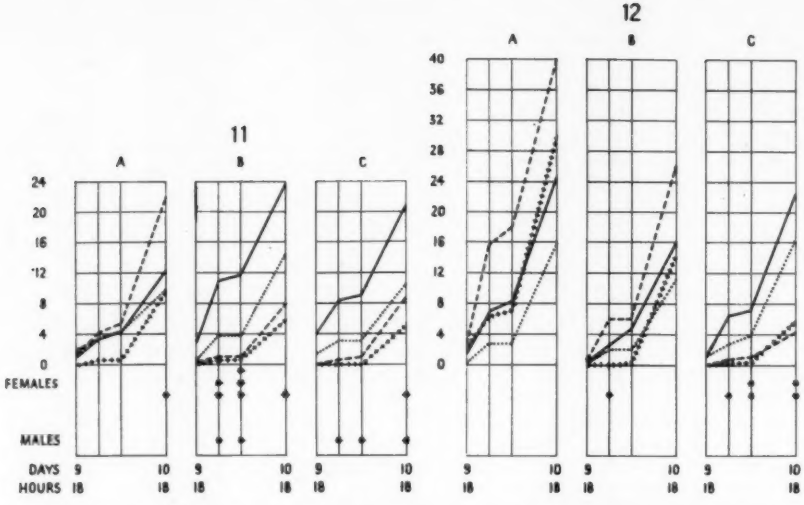
FIGURES 9 and 10.—Results from the competition experiment 1958:3 between pure strains with regard to early part of eclosion of flies which were autosomal homozygotes *a1/a1* (Figure 9) and autosomal heterozygotes *a1/a2* (Figure 10). Concerning the X chromosomes the curves show: A, solid line w^a females, broken line, wild type females, dotted line w^a males, cross-marked line wild type males; B, solid line w^a females, broken line w^{co} females, dotted line w^a males, cross-marked line w^{co} males; C, solid line w^a females, broken line w females, dotted line w^a males, cross-marked line w males. Each curve shows averages of three repeats. See also description in text.

TABLE 8

Experiment 1958:6

Total number of adults observed. Figures show totals from three vials per competition. Each vial started with 200 larvae of which 100 were taken from each of the two X chromosome strains shown in the table. Autosomal homozygotes are a2/a2; *autosomal heterozygotes are from crosses* a2/a2 ♀♀ × a1/a1 ♂♂

	Larvae from X chromosome strains shown below											
	w^a and +				w^a and w^{co}				w^a and w			
	w^a ♀♀	w^a ♂♂	+ ♀♀	+ ♂♂	w^a ♀♀	w^a ♂♂	w^{co} ♀♀	w^{co} ♂♂	w^a ♀♀	w^a ♂♂	w ♀♀	w ♂♂
Autosomal homozygotes	133	98	148	117	138	102	152	119	131	99	159	115
Autosomal heterozygotes	118	124	142	142	108	122	149	131	129	105	160	120



FIGURES 11 and 12.—Results from the competition experiment 1958:6 between pure strains with regard to early part of eclosion of flies which were autosomal homozygotes *a2/a2* (Figure 11) and autosomal heterozygotes *a2/a1* (Figure 12). Concerning the X chromosomes the curves show: A, solid line w^a females, broken line wild type females, dotted line w^a males, cross-marked line wild type males; B, solid line w^a females, broken line w^{co} females, dotted line w^a males, cross-marked line w^{co} males; C, solid line w^a females, broken line w females, dotted line w^a males, cross-marked line w males. Each curve shows averages of three repeats. The asterisks in Figure 12 refer to females. See also description in text.

TABLE 9

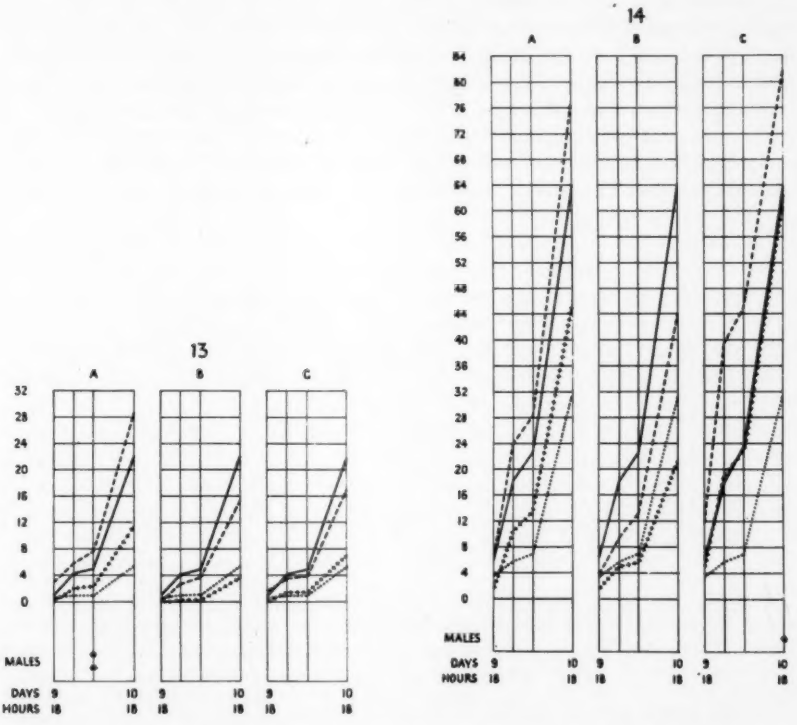
Experiment 1958:5

Total number of adults observed. Figures show totals from three vials with 200 larvae. Autosomal homozygotes are a2/a2; *autosomal heterozygotes are from crosses* a2/a2 ♀♀ × a1/a1 ♂♂

	Larvae from X chromosome crosses shown below							
	+/+ ♀♀ × + ♂♂		+/+ ♀♀ × w^{co} ♂♂		+/+ ♀♀ × w^a ♂♂		+/+ ♀♀ × w ♂♂	
Autosomal homozygotes	313	234	309	234	313	253	313	235
Autosomal heterozygotes	311	240	307	269	305	244	305	261

As the chief purpose of the experiments 1958:1 and 1958:2 was to compare the growth rates of females of different genotypes, these experiments were designed so as to always produce males of equal genotypical constitutions. This was done by always using X chromosome homozygous wild type females as mothers to the larvae, the growth rates of which were to be compared. By doing so all sons of these mothers were also wild type, and the sons were, hence, expected to be geno-

typically identical. However, very soon after the beginning of eclosion in experiment 1958:1, it was found that in the case of autosomal homozygosity, *a1/a1*, wild type males with w^a fathers grew at a higher rate than wild type males with fathers being +, w^{co} or w (Figure 15). At first it was believed that this was due to the Y chromosome: if so, we had, in the *a1/a1* strain of w^a, unintentionally isolated a special kind of Y chromosome. But we soon found that in the same experiment a corresponding thing also happened with the females: among the autosomal homozygotes, *a1/a1*, those females which, with regard to their X chromo-

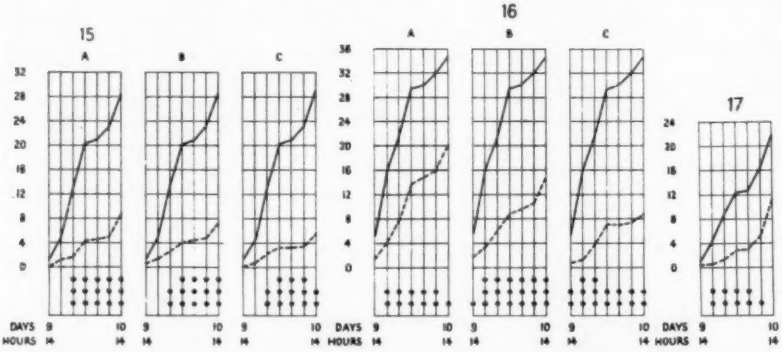


FIGURES 13 and 14.—Results from the noncompetition experiment 1958:5 with regard to early part of eclosion of flies which were autosomal homozygotes *a2/a2* (Figure 13) and autosomal heterozygotes *a2/a1* (Figure 14). Concerning the X chromosomes the curves show: A, solid line +/+ females, broken line $+/w^{co}$ females, dotted line wild type males from wild type fathers, cross-marked line wild type males from w^{co} fathers; B, solid line +/+ females, broken line $+/w^a$ females, dotted line wild type males from wild type fathers, cross-marked line wild type males from w^a fathers; C, solid line +/+ females, broken line $+/w$ females, dotted line wild type males from wild type fathers, cross-marked line wild type males from w fathers. Each curve shows averages of three repeats. See also description in text.

somes, were $+/w^a$, grew at a higher rate than any of $+/+$, $+/w^{co}$, or $+/w$ females (Figure 16).

When the identifications of the genotypes of the females in experiment 1958:2 were ready it was also found that *a1/a1* females which in their X chromosomes were $+/w^a$ grew at a higher speed in competition with any of the other types tested i.e. with any of $+/+$, $+/w^{co}$ or $+/w$ (Figures 3B, 5A, 5B). The wild type sons produced in experiment 1958:2 were always a mixture from fathers of two different kinds. Corresponding to the daughters shown in Figures 3 and 5 the fathers included w^a males in Figures 3B, 5A, and 5B but did not include w^a males in Figures 3A, 3C and 5C. The curves in Figure 17 show now the averages of the pooled number of males corresponding to these two groups of crosses respectively. As may be seen, here also male descendants of fathers including w^a males grew faster than descendants whose fathers did not include w^a males.

We have thus in experiments 1958:1 and 1958:2 found that, in combination with autosomal homozygosity *a1/a1* all descendants (female and male) of w^a males grew faster than descendants of $+$, w^{co} or w males. The w^a effect can consequently neither be believed to be located in the X, nor in the Y chromosome. As



FIGURES 15 and 16.—Results from the noncompetition experiment 1958:1 with regard to early part of eclosion of flies which were autosomal homozygotes *a1/a1*, Figure 15 males and Figure 16 females. The solid lines are in A, B, and C identical and show progeny from the X chromosome cross $+/+ \times w^a$; the broken lines show progenies from X chromosome crosses: A, $+/+ \times +$; B, $+/+ \times w^{co}$; C $+/+ \times w$. The curves of Figure 16 are the same as those shown in Figure 1, but arranged differently, so as to make other comparisons easier. Each curve shows averages of four repeats. See also description in text.

FIGURE 17.—Results from the competition experiment 1958:2 with regard to early part of eclosion of males which were autosomal homozygotes *a1/a1*. The solid line shows the pooled average number of males from three competitions, which, with regard to females, are shown in Figures 3 B, 5 A, 5 B, *viz.* progenies of $+/+ \times +$ and $+/+ \times w^a$; $+/+ \times w^{co}$ and $+/+ \times w^a$; $+/+ \times w$ and $+/+ \times w^a$. The broken line shows the pooled average number of males which, with regard to females, are shown in Figures 3A, 3C, 5C, *viz.* progenies of $+/+ \times +$ and $+/+ \times w^{co}$; $+/+ \times +$ and $+/+ \times w$; $+/+ \times w^{co}$ and $+/+ \times w$.

may be seen from Figures 2, 4, 6 no w^a effect was found in case of autosomal heterozygosity *a1/a2*. This also rules out the possibility of cytoplasmic inheritance as the cause of the w^a effect.

In the additional experiments with pure strains the w^a larvae of both sexes, which were autosomal homozygotes *a1/a1*, grew faster than other *a1/a1* larvae (experiment 3 Figure 9). But in the additional experiment with progeny testing of the females the X chromosome wild type homozygotes which were *a1/a1* grew at a higher rate than the $+/w^a$ females (experiment 4, Figure 7B); the difference was, however, of no statistical significance.

As mentioned earlier we have, in the synthesis of the strains, paid no attention to chromosome 4. It is thinkable that, by chance, one or several of the strains were rendered homozygous for chromosome 4 as well. Suppose now that that strain which was homozygous for the autosomal set *a1* and in its X chromosome contained the gene w^a, also was homozygous for a 4th chromosome dominant *A*, the effect of which was to accelerate larval growth rate. In such a case the progeny from all crosses within the *a1* strains—i.e., producing *a1/a1* homozygotes—in which the male parents were w^a, would carry the gene *A*, whereas the progeny from other kinds of males would not carry *A*. The hypothetical gene *A* would thus explain the results from experiments 1958:1 and 1958:2. A consequence of the existence of such a gene, *A*, would be that if the mothers were *a2/a2* a w^a effect would show up among the larvae, only if the fathers were *a1/a1* but not if they were *a2/a2*. The results from the additional experiments, however, do not speak in favour of the existence of such a dominant gene *A*, as may be seen from Figures 8D, 11B, 11C, 12A, 12B, 13, 14. But as we believe that the general background of growth rate is to be found in a great number of polygenes one must expect that the interaction between a major gene *A* and the polygenes is of a very complicated nature. As the purpose of making the present studies was of a quite different kind we did not find it worth while to inquire more into the causes of the w^a effect. We may thus content ourselves by saying that the w^a effect perhaps is due to a dominant gene in chromosome 4, but that we have not been able to prove that this is the case.

The main problem: Viability expresses itself, and may be measured, in a number of different ways. Likewise, selection acts on several different characters, and may exert its influence during many different stages of an animal's development. In most animals such characters as fertility and fecundity are of a high selectional importance. The same is the case with abilities for search for food in quantities enough for maintenance and growth. Most of that part of the food which is used for growth in holometabolic insects is consumed during the larval period. Now, it is known, that larval growth rate in Drosophila is to a large extent genetically determined (DOBZHANSKY, HOLZ and SPASSKY 1942; BONNIER and JONSSON 1957; MARIEN 1958). That this rate, besides external influences, must be strongly related to larval competition abilities seems, moreover, evident. We think, hence, that when we use larval growth rate and larval competition ability—measured

by the time which elapses from the mother's oviposition to the moment her offspring reaches eclosion—we have chosen a character which from the point of view of viability and selection is of a very high importance.

When, in the 1957 series, wild type homozygous females +/+ were crossed to +. w^{co}, w^a, w^t, or w males and thus made competitions possible between wild type homozygous female larvae +/+ and any of the wild type heterozygous larvae viz., $+/w^{co}$, $+/w^a$, $+/w^t$, or $+/w$ (in which cases the competing male larvae always were wild type) it was in all cases found that the homozygous female larvae grew at a higher rate than the heterozygous ones. These experiments (experiment 1957:3, Table 2) were, however, made on a very small scale. The chief reason for starting the 1958 series of the present study was the hope, thereby, to get some more knowledge of the influence of homozygosity and heterozygosity on the rate of larval development.

We have now results permitting two kinds of comparisons *viz.*, on the one hand, the effect of autosomal homozygosity and heterozygosity on the growth rate of larvae of both sexes; and, on the other hand, the effect on female larvae of homozygosity and heterozygosity in the X chromosome.

Autosomal homozygosity and heterozygosity: The difference in growth rate between autosomal homozygotes and autosomal heterozygotes is in most cases of the present study very conspicuous and does not make any statistical treatment necessary. Autosomal homozygotes *a1/a1* and heterozygotes *a1/a2* may be compared on several of the diagrams: Figure 1 versus Figure 2, Figure 3 versus Figure 4, Figure 5 versus Figure 6, Figure 7A, B, C, versus Figure 8A, B, C. The differences between the curves of the pure strains Figure 9 versus Figure 10 may seem less obvious, but if one disregards the w^a effect and only compares the curves relating to the non w^a larvae the difference is again found to be very conspicuous. Comparisons between *a2/a2* homozygotes and *a2/a1* heterozygotes may be made on diagrams, Figure 7D versus Figure 8D, Figure 13 versus Figure 14. The differences between the curves of the pure strains Figures 11 and 12 are less obvious, but if one again only compares the non w^a larvae one will find a higher growth rate in Figure 12A, B, than in Figure 11A, B. Only with regard to the w larvae one does not find a higher growth rate of the heterozygotes *a2/a1* than of the homozygotes *a2/a2* (Figures 11C and 12C).

We thus have in the great majority of our experiments found a higher viability —expressed as a higher larval growth rate—among autosomal heterozygotes than among autosomal homozygotes. Now, the synthesis of our homozygous strains has most probably also included the homozygotization of one or more detrimental genes. From this point of view one could perhaps formulate the results by saying that it is not heterozygosity which enhances viability but homozygosity with regard to detrimentals which reduces viability. If the low viability of the homozygotes were due to detrimentals, the much higher viability of the heterozygotes shows that the dominance effect, if any, of these detrimentals must be supposed to be of a small magnitude. At any rate, such homozygotes as our *a1/a1* or *a2/a2* individuals are most certainly, so to say, unnatural creatures, and we think that

our results rather strongly indicate that selection must, in *D. melanogaster*, favour autosomal heterozygotes.

Sex-linked homozygosity and heterozygosity: With regard to our comparisons between X chromosome homozygotes and heterozygotes the results point more or less in the opposite direction when compared to autosomal homozygotes and heterozygotes. The differences are, however, never as conspicuous in the former comparisons as in the latter.

When looking at the diagrams with the aim of comparing X chromosome homozygotes and heterozygotes, one must disregard the w^a effect. One finds then, in the main experiments 1958:1 and 1958:2, that in combination with autosomal heterozygosity *a1/a2*, the wild type homozygous female larvae grew at a higher rate than any of the heterozygotes $+/w^{co}$, $+/w^a$, or $+/w$ (Figures 2 and 4) and that the differences were of a more or less good statistical significance. Also in the case of autosomal homozygosity *a1/a1* the wild type homozygotes were found to grow faster than the heterozygotes $+/w^{co}$ and $+/w$, though without statistical significance in experiment 1958:1 (Figure 1A, C) but with some significance in experiment 1958:2 (Figure 3A, C).

From the point of view of X chromosome homozygosity and heterozygosity the results of the additional experiments were rather erratic, as may be judged directly from Figures 7, 8, 13, 14. As mentioned in the description of the experimental technique of these experiments, they could not be performed with all the precautions taken in the 1958:1 and 1958:2 experiments. Non random environmental influences on larval growth rate which probably never can be wholly avoided may, therefore, be believed to have been more influential in the additional experiments than in the main experiments.

We find it valid to lay more emphasis on the results of the main experiments than on the additional experiments, and it seems, hence, safe to conclude that female larvae which in their X chromosomes are homozygous for a wild type allele and of the same general constitution as our homozygotes, usually, but not always, have a higher viability than those which are X chromosome heterozygotes. As the higher viability of the X chromosome homozygotes in the 1958:1 and 1958:2 experiments was somewhat more marked in combination with autosomal heterozygosity (Figure 2 versus Figure 1 and Figure 4 versus Figure 3) one may suppose that it is easier for the viability promoting nature of X chromosome homozygosity to be expressed if it has the background of the viability enhancing effect of autosomal heterozygosity than if it lacks this background.

When using the results of the present studies in order to try to evaluate the importance of heterozygosity versus homozygosity and to compare the effects of sex-linked genes with autosomal genes, one must remember that the part of the X chromosome which in our experiments was heterozygous was confined to a section of a maximal length of 4.7 map units while the autosomal heterozygosity included the total length of the two long autosomes. If an individual is heterozygous in one single locus and if this individual from some point of view is more viable or more luxuriant or, in general, has to be placed further away on the plus

side of the scale than any of the two homozygotes, one speaks of overdominance. Now, the X chromosomes of our strains could, of course, not be synthesized in such a way as to make the heterozygous part of the heterozygotes be confined to one single locus. But if there really was overdominance for some of the heterozygotes in the *w* locus, then it seems to be evident that also the wild type heterozygotes of the actual constitution as they are in our experiments, should show a higher viability than the wild type homozygotes. As this was not the case we must conclude that our results—as far as they go—do not indicate any kind of overdominance in the *w* locus. As is well-known, several investigations have been made on heterosis in maize. Quite recently JONES (1957) compared plant heights in lines which were either homozygous or heterozygous for two specified loci, but he found no sign of overdominance.

In our experiment 1957:1, Table 1, it was found that the recessive strains w^{co}, w^a, w^t and *w* had a lower viability than the wild type strain. In combination with autosomal heterozygosity a corresponding result was found with regard to w^a (the only recessive tested from this point of view in the 1958 series) in experiments 1958:3 (Figure 10A) and 1958:6 (Figure 12A). MULLER has on several occasions expressed the opinion that most recessives of a low viability show dominance effects, in the sense that their heterozygotes with wild type alleles show a lower viability than wild type homozygotes. From this point of view our results with the *w* alleles fit moderately well with MULLER's opinion. He also pointed out (1956) that "the exceptional cases of the heterozygote being superior are probably represented for the most part . . . by adaptations that have not yet stood the test of geological time". With regard to such special cases as those studied by us it seems also, in accordance with MULLER's views, probable that when such genes as the recessives of the *w* allelic series are found in nature, they are the result of recent mutations.

Concerning the results of the present investigation one may in this connection make the following three points: (1) There is in Drosophila, an important difference in the possibilities for selection to act upon, on the one hand, sex-linked, and, on the other hand, autosomal genes. In wild populations, as well as in mass propagated stock bottles, natural selection must, via the hemizygous males, strongly act in the direction to remove X chromosomes with detrimental genes. Such an action would be counterbalanced only if females heterozygous for the detrimentals had a much higher fecundity than the nondetrimental homozygotes. An action of natural selection against autosomal detrimentals may also be at work but certainly much more indirectly and at a much lower pace. (2) The genes, used in our experiments, besides being visibles in homozygous conditions, were all recessives, and it is not known what the results would have been if our heterozygotes had been heterozygous for different wild type alleles. (3) Our heterozygotes were, in the X chromosomes, heterozygous for very short chromosome sections, but in the autosomes heterozygous for the whole of these chromosomes, i.e. for very long chromosome sections. Now, it does not seem wholly unreasonable to imagine that —even if heterozygotes for one wild type and one recessive gene are less viable

than the wild type homozygotes, when the loci are studied separately—the heterozygosity *per se* would increase the viability of the heterozygotes above that of the wild type homozygotes if one studies several loci simultaneously, i.e. if one studies chromosome sections of a moderate length. It is our intention to try to elucidate these points somewhat more in a new set of experiments.

In this connection the following remark may be made. It is commonplace to emphasize that the different genes of the genome cooperate during development, or to stress that the effects of a certain gene pair depend upon the whole genetic background. A comparison of the curves in Figures 1 and 2, 3 and 4, 5 and 6 give as it seems to us, an unusually strong impression of this fact: the same sex-linked genes have very different effects when associated with the one or the other of the autosomal gene combinations. In genetic class work, when one, for instance, has to demonstrate MENDEL's law of segregation, it is necessary to speak in terms of single genes. Also, when one is studying such difficult problems as mutation frequencies or the mutational process itself, it may be necessary to concentrate the observations on circumstances within single genes or gene pairs. A lot of other examples could easily be given in which it is obviously legitimate to base the discussion on the happenings within single gene pairs. The situation may, however, be different in other cases. In the mathematical treatment of population genetics, one may solve the equations only if he confines and states the problems in terms of single gene pairs. However, we think that it is necessary to be very cautious when discussing or making experiments within the field of experimental population genetics: to confine the discussion to single gene pairs may be a gross simplification of the actual situation; a simplification which easily may lead to quite erroneous conclusions.

There is one more point which we have not mentioned. As may be seen from several of the tables the total number of females in the progeny from crosses between *a1/a1* females and *a2/a2* males was significantly more than 50 percent of the total number of larvae collected. We are trying to investigate this problem somewhat more.

SUMMARY

1. The material for the present study consists of a number of strains which, with regard to the X chromosomes, were made identical except for a short region including the white locus, in which they contained different *w* alleles. These X chromosomes were combined with the one or the other of two different wild type sets of the two long autosomes.

2. In all experiments 200 freshly hatched larvae were put into each of a number of food containing vials. Most of the experiments were performed as larval competition experiments, in which 100 larvae from two different sources competed with each other. Eclosion was checked at several instances before it ceased, and so the relative speed with which the competing types reached eclosion could be followed.

3. The results showed, *inter alia*, that larvae which were heterozygous with regard to the autosomal sets usually grew conspicuously faster than those which

were homozygous with regard to the autosomes. Female larvae which were wild type homozygous with regard to the X chromosomes usually grew faster and had a higher competing ability than those which, in the *w* locus containing segment, were heterozygous for one wild type and one nonwild type *w* allele. Thus, no signs of overdominance were found when a wild type allele was combined in a heterozygote with a recessive *w* allele.

4. In the earlier part of the experiments the competing ability of larvae of the pure strains indicated a seriation following the degree of deviation from normality expressed as deviation in eye color from the wild type red color.

ACKNOWLEDGEMENTS

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CHIASMA TERMINALIZATION IN A STRUCTURAL HETEROZYGOTE IN SECALE

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TERMINALIZATION of chiasmata which originate in structural heterozygotes between centromeres and points of non-homology is said to be arrested at such points. The hypothesis that terminalization stops at points where chromosome homologies change (DARLINGTON 1929) is based on two assumptions: (1) That point-by-point attractions which cause homologous chromosome segments to conjugate in pairs continue to exert a force through late prophase and that after chromosomes are effectively doubled the attractions hold chromatid pairs together rather than chromosome pairs; and (2) that attractions of like chromatids cannot be overcome by terminalization which, should it proceed beyond a point where chromosome homologies change, would cause a change from association of identical chromatids to an association of unlike chromatids. However, contradictions to the hypothesis have been reported by MARQUARDT (1948) and by HAGBERG (1954). The contradiction reported by HAGBERG was based on observations of certain chiasmata in heteromorphic bivalents, but no assurance was given that the chiasmata originated in segments proximal to changes in homology. MARQUARDT, on the other hand, observed multiple terminal chiasmata in translocation configurations, which clearly required the interpretation that chiasmata originated at interstitial sites and moved through non-homologous segments to terminal positions.

While studying meiosis in pollen mother cells of *Secale cereale* L. × *S. montanum* Guss. hybrids, the writer observed a translocation association comparable with those described by MARQUARDT. The association occurred in *r* hybrid No. 168–1, a plant obtained by pollinating *S. cereale* with X-rayed pollen of *S. montanum*. A first metaphase cell showed a 5-chromosome chain, a 4-chromosome "frying-pan-shaped" association, two bivalents, and one univalent (PRICE 1955, Figure 8). The univalent and the 5-chromosome chain resulted from naturally occurring structural differences—reciprocal translocations—which distinguish the chromosomes of the two species (SCHIEMANN and NURNBERG-KRUGER 1952; PRICE 1955; RILEY 1955; NAKAJIMA 1956; STUTZ 1957). The "frying-pan" configuration resulted from heterozygosity for an induced interchange between chromosomes not involved in naturally occurring translocations. This 4-chromosome induced-translocation configuration has special significance relative to the hypothesis that chiasma terminalization stops where chromosome homologies change.

The significance of the "frying-pan" configuration lies in its 3-chromosome terminal chiasma (Figure 2). A 3-chromosome terminal chiasma at first metaphase presupposes early prophase chiasmata in both segments of a chromosome arm which is paired in one segment with a second chromosome and in another segment with a third chromosome. In a translocation heterozygote such an association shows that chiasmata originate in segments both distal and proximal to the point of interchange. As Figure 1 illustrates, a completely terminalized 3-chromosome connection in a translocation configuration must result from movement of a chiasma through a point where chromosome homologies change.

Having recently reviewed MARQUARDT's contribution, the writer was prompted

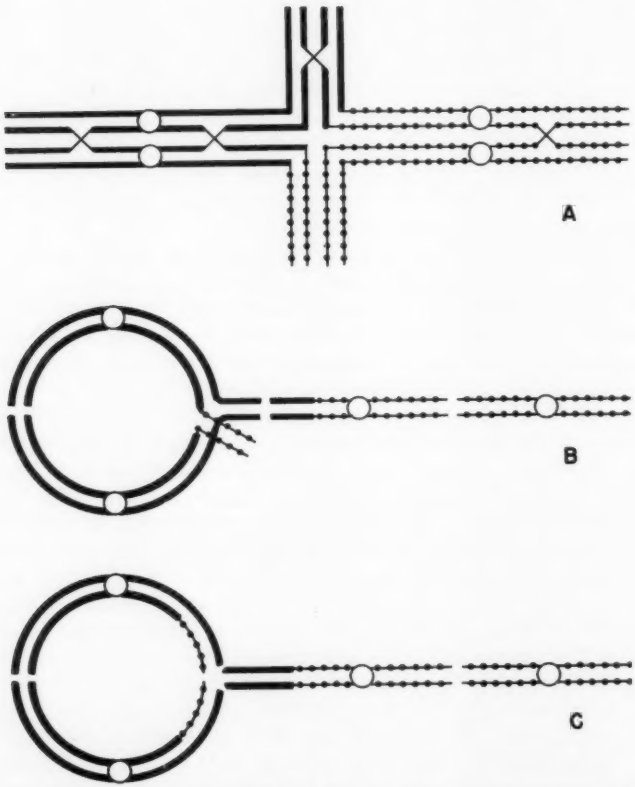


FIGURE 1.—Diagrams of 4-chromosome associations in a translocation heterozygote with points of interchange in median positions: A, Pachytene association with chiasmata located so that a "frying-pan" configuration would result at first metaphase; B, first metaphase with terminalization of proximal chiasma arrested at the point of interchange; C, first metaphase with terminalization completed despite changes of chromosome homology.

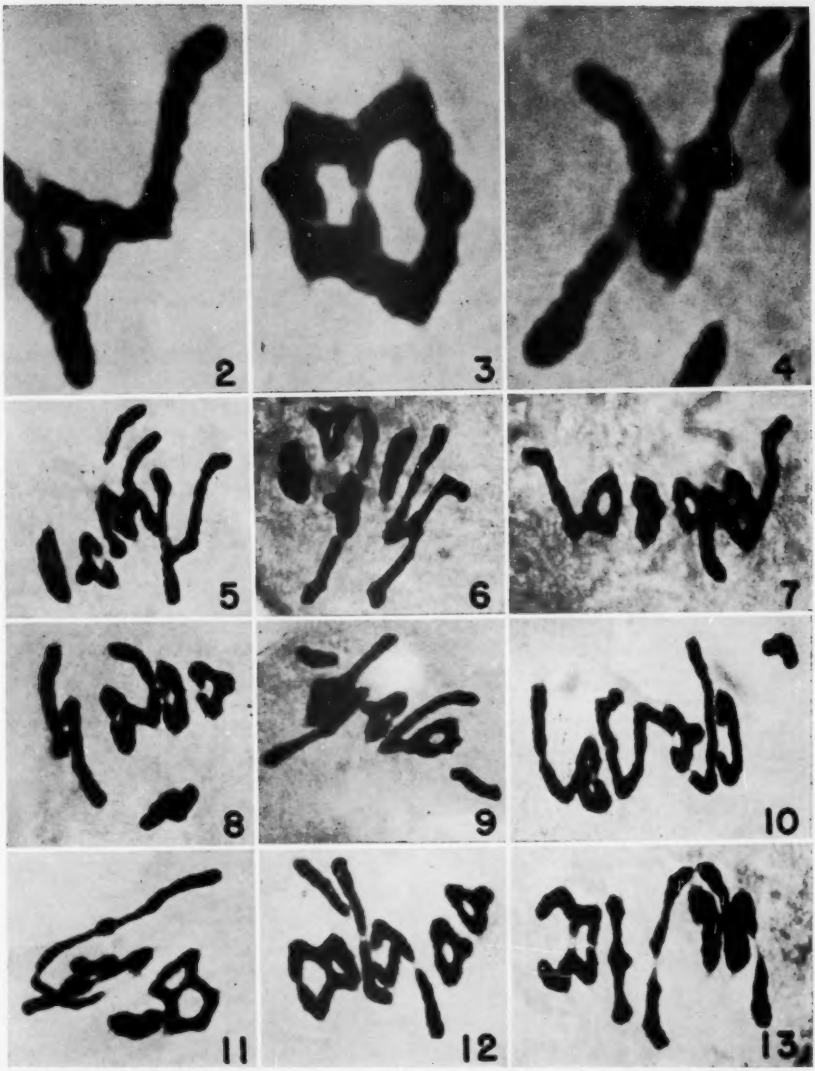


FIGURE 2.—A 4-chromosome "frying-pan" configuration at first metaphase showing a completely terminalized 3-chromosome chiasma. × 3200.

FIGURE 3.—A 4-chromosome "figure-of-eight" at first metaphase. × 3200.

FIGURE 4.—A 4-chromosome "Y-shaped" configuration at first metaphase. × 3200.

FIGURES 5 and 6.—First metaphase cells with "Y-shaped" configurations. × 1600.

FIGURES 7, 8, 9, and 10.—First metaphase cells with "frying-pan" configurations. × 1600.

FIGURES 11, 12, and 13.—First metaphase cells with "figures-of-eight. × 1600.

to return to permanent preparations of pollen mother-cell divisions in *r*-hybrid No. 168–1. The permanent slides were re-examined, particular attention being given to the behavior of the four chromosomes involved in the induced translocation association.

In Table 1, diagrams show first metaphase configurations that could be expected if chiasma formation were variable in the several segments of a translocation association such as the one illustrated in Figure 1-A. The diagrams show interstitial chiasmata only where terminalization might be arrested by homology changes. These diagrammed expectancies can be compared with configurations actually formed by the four chromosomes involved in the induced translocation association.

OBSERVATIONS

Configurations formed in the first meiotic metaphase by the four chromosomes under investigation and the frequencies in which they were observed are shown in Table 2. In all, 482 cells in which the chromosome associations could be interpreted were found; in 172 of these cells, however, the four chromosomes of the induced translocation association could not be distinguished from the six chromosomes involved in the naturally occurring translocation association.

TABLE 1

Diagrams of first metaphase configurations expected if chiasmata fail in certain segments of four-chromosome translocation associations

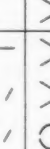
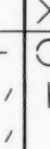
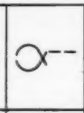
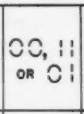
DISPOSITION OF CHIASMATA IN STRUCTURALLY HOMOZGOUS ARMS AND IN DISTAL SEGMENTS OF HETEROZYGOUS ARMS	DISPOSITION OF CHIASMATA IN PROXIMAL SEGMENTS OF THE STRUCTURALLY HETEROZYGOUS ARMS				
	CHIASMATA LACKING IN PROXIMAL SEGMENTS	A CHIASMA IN ONLY ONE PROXIMAL SEGMENT		CHIASMATA IN BOTH PROXIMAL SEGMENTS	
		ARRESTED	TERMINAL	ARRESTED	TERMINAL
CHIASMATA IN BOTH HOMOZYGOUS ARMS AND IN BOTH DISTAL SEGMENTS					
CHIASMATA IN BOTH HOMOZYGOUS ARMS BUT LACKING IN ONE OR BOTH DISTAL SEGMENTS					
CHIASMATA IN BOTH DISTAL SEGMENTS BUT LACKING IN ONE OR BOTH HOMOZYGOUS ARMS					
CHIASMATA LACKING IN ONE OR BOTH HOMOZYGOUS ARMS AND LACKING IN ONE OR BOTH DISTAL SEGMENTS					

TABLE 2

*Frequencies with which the induced translocation association forms various configurations in first metaphase cells**

								 OR	UNCERTAIN
18	17	5	5	2	4	136	1	122	172

* Here the distinction between complete and incomplete terminalization is made only for "frying-pan" and "Y-shaped" configurations.

Chromosome associations in 122 other cells showed that the four chromosomes of the induced-translocation association formed two bivalents, but it was uncertain whether one bivalent or both were open or whether one or both were closed. In 136 cells the four chromosomes definitely formed two closed bivalents, and in one cell they formed two open bivalents. The induced translocation association produced 4-chromosome configurations in 51 cells. These 4-chromosome associations were in the forms of rings, "frying-pans," "figures-of-eight," and "Y-shaped" configurations (Figures 2–13). No univalents or 3-chromosome associations could be attributed to the four chromosomes under investigation.

Many of the configurations theoretically possible were not observed. The absence of univalents, 3-chromosome configurations, and certain types of 4-chromosome configurations indicates that chiasmata rarely failed in the structurally homozygous arms. Variable chiasma failure did occur in the distal and proximal segments of the heterozygous arms, however.

The "figures-of-eight" (Figures 3, 11, 12, 13) resembled a figure "8" only roughly. The induced reciprocal translocation was probably asymmetrical, and very likely this asymmetry was responsible for the appearance of the "figures-of-eight." They were not the kind of configuration expected if chiasmata in proximal segments were arrested at points of non-homology; neither could these configurations be interpreted as having completely terminalized 4-chromosome chiasmata. Because of this difficulty, arrested chiasmata are not distinguished from terminal multiple chiasmata in listing "figures-of-eight" in Table 2.

Five "frying-pan" configurations were seen with completely terminalized 3-chromosome chiasmata, but none of them could be so clearly photographed as the configuration first described (Figure 2). The multiple connections in 17 other "frying-pan" configurations were incompletely terminalized or were obscure. Two of the seven "Y-shaped" configurations had completely terminalized 3-chromosome chiasmata.

When two of the four chromosomes of the induced translocation association form a closed bivalent, a chiasma must have originated in the homologous proximal segments of chromosome arms whose distal segments are non-homologous. Complete terminalization in both arms of such a bivalent requires movement of

a chiasma past a point where homologies change. Two cells which provided critical evidence of this sort were observed. The first cell contained a 6-chromosome translocation chain (the naturally occurring translocation association) and four closed bivalents. In three of the closed bivalents the chiasmata in both arms were completely terminalized. The second cell contained a 5-chromosome chain (part of the naturally occurring association), three closed bivalents, one open bivalent, and a univalent. Terminalization was complete in both arms of all three closed bivalents. Thus, in each of these cells, there was at least one closed and completely terminalized bivalent composed of chromosomes that differed in the distal segments of one arm.

DISCUSSION

After terminalization had been demonstrated in several species, an explanation was sought for chiasmata in other species that appeared to be localized or failed to terminalize completely. DARLINGTON (1929) recognized that genic and environmental influences might control terminalization in some cases but suggested that incomplete terminalization might also result from the arrest of chiasmata at points where homologies change. Later, DARLINGTON (1931) supported his suggestion with observations of interstitial chiasmata at first metaphase in Oenothera translocation heterozygotes. Terminalization in Oenothera is usually complete, but concurrence of structural hybridity and persistent interstitial chiasmata should not have been regarded as conclusive supporting evidence for his hypothesis. Nevertheless, this case was cited later (DARLINGTON 1937, p. 511) as an "established" example of the arrest of terminalization by change of homology. Of the other "established" cases cited, only SANSOME's (1932) observations in Pisum provided any real support for DARLINGTON's hypothesis. When a 6-chromosome translocation association formed "figures-of-eight" in SANSOME's material, the interstitial chiasmata seemed to occur in a constant position at first metaphase. This suggested to SANSOME that terminalization had been stopped at a point where homologies changed.

The other "established" cases cited by DARLINGTON are much less conclusive. CATCHESIDE (1935) illustrated an interstitial chiasma in a "frying-pan" translocation association in X-rayed Oenothera but made no point of interpreting this configuration. CATCHESIDE's earlier study (1932) of haploid Oenothera and ERLANSON's (1931) study in Rosa were also cited as "established" cases; however, both of the authors accepted DARLINGTON's hypothesis as their basic tenet and interpreted their observation in the light thereof. It is interesting that in both cases completely terminal multiple chiasmata were described and illustrated in configurations which, it appears, should have been recognized as translocation associations. However, these configurations were not recognized as contradicting DARLINGTON's hypothesis. Instead, the validity of the hypothesis remained unquestioned, and the configurations were interpreted as evidence for segmental duplication.

DARK (1936) in a study of Paeonia and DARLINGTON and GAIRDNER (1937)

in a study of Campanula made similar interpretations. Translocation configurations were described in both materials; and when incompletely terminalized chiasmata were observed in these configurations, they were ascribed to the interruption of terminalization at non-homologous points. Completely terminalized 4-chromosome chiasmata were found in both studies; but in spite of the translocation configurations in the same materials, the 4-chromosome terminal chiasmata were regarded as evidence for duplicated terminal segments. DARLINGTON and GAIRDNER asked themselves why the four duplicated chromosome ends did not regularly pair, and answered that the parts assumed to be in common must have been very small. Such an interpretation requires that three chiasmata involving four chromosomes originate in a very small chromosome segment, an unlikely event.

Possibly non-homology arrests terminalization in certain cases. SANSOME's observations in Pisum seem to provide an example. However, the general validity of the idea that changes in homology stop terminalization must be rejected. One reaches this conclusion not only because of the contradicting evidence presented first by MARQUARDT and again in the present article, but also because of the inconclusiveness of the evidence on which the hypothesis was formed.

SUMMARY

Heterozygosity for an induced reciprocal translocation in a *Secale cereale* L. × *S. montanum* Guss. hybrid resulted in first-metaphase configurations which show that the point of translocation is so located that chiasmata form both proximal and distal to it. Several of the configurations exhibit terminal multiple chiasmata. Such configurations refute the hypothesis that terminalization of chiasmata is arrested at points where homologies change. Literature supporting the hypothesis is reviewed and found to be wanting in conclusive evidence.

ACKNOWLEDGMENT

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INHERITANCE OF QUANTITATIVE CHARACTERS IN ZEA MAYS. I. ESTIMATION OF THE NUMBER OF GENES CONTROLLING THE TIME OF MATURITY

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THE estimation of the number of gene pairs controlling quantitative characters is a great challenge to geneticists. While the study of discontinuous variations is relatively simple, that of continuous variations is complicated, since such a study is concerned with multiple factors, heterosis, dominance and the nature of the interactions between the genes differentiating these quantitative characters.

The method suggested by ANDERSON (1938) using chromosomal interchanges as tools for detecting the number of gene pairs controlling quantitative characters, and applied by BURNHAM and CARTLEDGE (1939), SABOE and HAYES (1942), MILLER (1951), IBRAHIM (1954) and MOHAMED (1954, 1956), will not reveal the type of gene interactions. The chromosomal interchanges used in such studies were not in an isogenic background, which limits the final conclusions. Therefore, the methods given by EMERSON and EAST (1913) and POWERS (1950a) are very adequate if the genetic and environmental variabilities are following the same scale of measurement and the gene effects are additive on such a scale.

POWERS, LOCKE and GARRETT (1950) suggested and described a method for estimating the number of genes controlling quantitative characters. This method is based on partitioning the backcrosses and F_2 populations into their component genotypes. Also it involves the separation of environmental and genetic variances from the total variances with the purpose of determining the nature of all these variances. This can be accomplished from the three nonsegregating populations, namely, P_1, F_1, and P_2, and the three segregating populations, B_1 to P_1, F_2, and B_1 to P_2. The latter are essential in arriving at the entire genetic hypothesis.

The study in this paper will be limited to the estimation of the number of genes controlling the number of days from seeding to silking and pollen shedding. Also the number of gene pairs differentiating the two parents for the difference of days between silking and pollen shedding will be discussed. The time of maturity was used in these studies because of its importance in the production of hybrid corn, since differences between inbreds with respect to this character will affect the general output of the yield.

MATERIAL AND METHOD

The hybrid populations were obtained from a cross between E.G. 102 (P_1), and E.G. 205 (P_2) maize inbred lines, which were kindly furnished to the author by DR. M. A. IBRAHIM of the Ministry of Agriculture, Egypt, U.A.R. The genetic

study of the experiment included P_1, B_1 to P_1, F_1, F_2, B_1 to P_2, and P_2 populations. The field design of the experiment was a randomized complete block. Planting of the experiment started in the summer of 1955, at the University of Alexandria Experimental Farm. The above two inbreds were selected for this study because they are considered very promising in the Ministry of Agriculture program for producing hybrid corn for commercial use.

The dates of silking and pollen shedding were recorded once daily on the same plant. The means and the standard deviations were calculated to study the phenotypic dominance. The environmental and genetic variances were estimated according to the method suggested by Powers (1942), and Powers *et al.* (1950). In testing for the proper scale, the method discussed by Powers *et al.* (1950) and Powers (1951) was used.

EXPERIMENTAL RESULTS

1. Silking date

One of the essential steps in studying any continuous character is to determine the type of scale of measurement which fits better the biological processes causing the variability.

It can be seen from Table 1 that neither the arithmetic scale (on an additive base) nor the logarithmic scale agreed with the obtained means. The mean values for the number of days from seeding to silking date showed that E.G. 102 (P_1) had a mean of 65.73 days and E.G. 205 (P_2) a mean of 67.68 days. The difference between the two inbred lines was 1.95 days with a P value of 0.01. The mean of E.G. 102 (P_1) was slightly higher than that of the F_1 (64.95 days), but the difference was within the limits of variation expected due to the errors of random sampling. The mean of B_1 to P_1 (64.19 days) was less than the mean of P_1, with a difference of 1.54 days. This difference was significant. However, χ^2 test showed that the frequency distribution of B_1 to P_1 was not significantly different from that of P_1 and F_1, since χ^2 value was 13.0179 with a P value of .20–.10 (d.f. 8). Also "t" tests indicated that the mean of the F_1 did not differ significantly from the mean of B_1 to P_1. From the above analysis it was concluded that phenotypic dominance was complete for fewer days over more days.

TABLE 1

The obtained and the calculated means on the basis of both arithmetic and logarithmic scales for the number of days from seeding to silking

Generation	$\bar{X} \pm$ S.D.	Arithmetic mean	Logarithmic mean	Number of individuals
P_1	65.73 ± 3.05	65.73	65.73	40
B_1 to P_1	64.19 ± 2.47	65.34	65.34	122
F_1	64.95 ± 3.33	66.70	66.69	39
F_2	65.19 ± 3.12	64.09	64.09	154
B_1 to P_2	63.99 ± 3.04	66.31	66.29	147
P_2	67.68 ± 2.81	67.68	67.68	40

The fact that the genetic variance (Table 2) of B_1 to P_1 was not significant and was very small compared with that of F_2 and B_1 to P_2, indicated that genic dominance was also complete.

Number of major gene pairs differentiating parents: On the basis of the segregating generations, the assumption was made that the two parents were differentiated by three major gene pairs. The indication that three major gene pairs were involved was arrived at from the fact that only 1.95 percent of the individuals in the F_2 population fell in the class 65–69, whereas on a three-factor basis, 1.6 percent would be expected (Table 3). Also the indication was further supported by dividing 0.68 percent (class 72–75 of B_1 to P_2) by 7.70 percent (class 72–75 of F_1) which gave a value of 8.83 percent. On a three-factor basis, a ratio of 12.5 percent would be expected.

When the theoretical means of F_2 and B_1 to P_2 for one factor pair of genes, two pairs and three pairs were compared with the obtained means of these two populations (Table 4), it appeared that the calculations based on the hypothesis that the two inbred lines were differentiated by three major gene pairs was probably the best fit. Accordingly, the genotype of P_1 (E.G. 102) was symbolized as *AABBCC* and of the P_2 (E.G. 205) as *aabbcc*.

The B_1 to P_1 and B_1 to P_2 frequency distributions were partitioned into their component genotypes (Table 5). This partitioning will give the frequency distri-

TABLE 2

The means, standard deviations, variances and number of individuals in each population for the number of days from seeding to silking

Population	$\bar{X}$	S.D.	Total variance	Environmental variance	Genetic variance	Number of individuals
P_1	65.73	3.05	5.788	6.345	— .557	40
B_1 to P_1	64.19	2.47	4.581	6.580	—1.999	122
F_1	64.95	3.33	6.485	6.453	.033	39
F_2	65.19	3.12	9.538	6.430	3.108	154
B_1 to P_2	63.99	3.04	7.874	6.610	1.264	147
P_2	67.68	2.81	6.586	6.051	.535	40

TABLE 3

Condensed frequency distribution for the number of days from seeding to silking in percentages

Population	Classes in percentages					Number of individuals
	56–59	60–63	64–67	68–71	72–75	
P_1	2.50	17.50	55.00	22.50	2.50	40
B_1 to P_1	2.46	40.17	49.18	8.20	.....	122
F_1	5.13	30.77	43.59	12.83	7.70	39
F_2	1.95	30.51	46.11	16.89	4.54	154
B_1 to P_2	5.44	38.78	45.58	9.52	.68	147
P_2	.....	10.00	35.00	45.00	10.00	40

TABLE 4

Theoretical values for the number of days from seeding to silking based on hypothesis that indicated the number of gene pairs differentiating the two inbreds

Number of gene pairs	Mean for F_2	Mean for B_1 to P_2
1	69.1	66.70
2	65.82	69.10
3	65.65	66.95
Actual	65.19 ± 3.12	63.99 ± 3.04

TABLE 5

Partitioning of B_1 to P_1 and B_1 to P_2 frequency distribution into their component genotypes for the number of days from seeding to silking

Population and genotype	Row no.	Classes in percentages 56–59	60–63	64–67	68–71	72–75	Theoretical percentage
B_1 to P_1	1	2.46	40.17	49.18	8.20	.....	100.00
$F_1 + P_1$ (*AaBbCc* + *AABBCC*)	2	.95	6.03	12.32	1.28	4.42	25.00
Row 1 — row 2	3	1.51	34.14	36.86	6.92	—4.42	75.00
Row 3 on positive basis	4	1.51	34.14	36.86	2.50	.....	75.00
Row 4 (100% basis)	5	2.01	45.52	49.15	3.33	.....	100.00
F_1 (*AaBbCc*)	6	5.13	30.77	43.59	12.83	7.70	12.50
P_1 (*AABBCC*)	7	2.50	17.50	55.00	22.50	2.50	12.50
B_1 to P_2	8	5.44	38.78	45.58	9.52	.68	100.00
$F_1 + P_2$ (*AaBbCc* + *aabbcc*)	9	.64	5.10	9.82	7.23	2.21	25.00
Row 8 — row 9	10	4.80	33.68	35.76	2.29	—1.53	75.00
Row 10 on positive basis	11	4.80	33.68	35.76	.76	.....	75.00
Row 11 (100% basis)	12	6.40	44.91	47.68	1.01	.....	100.00
F_1 (*AaBbCc*)	13	5.13	30.77	43.59	12.83	7.70	12.50
P_2 (*aabbcc*)	14	.....	10.00	35.00	45.00	10.00	12.50

bution for the different genotypes in the B_1 to P_1 and B_1 to P_2 populations. Following the partition, a theoretical frequency distribution for the F_2 genotypes could be calculated. Twenty-seven genotypes would be expected in the F_2 generation. However, the following genotypes of the F_2 do not occur in either of the two backcross populations.

Group I

AA	*BB*	*cc*	*Aa*	*BB*	*cc*
AA	*Bb*	*cc*	*Aa*	*bb*	*CC*
AA	*bb*	*CC*	*aa*	*BB*	*CC*
AA	*bb*	*Cc*	*aa*	*Bb*	*Cc*

Group II

AA	*bb*	*cc*		*AA*	*bb*	*cc*
aa	*BB*	*cc*				

It can be seen that group I had two nonallelic dominant genes, while group II had only one dominant gene. The frequency distribution of the group I genotypes could be calculated by taking 66.67 percent of the P_1 distribution and 33.33 percent of the P_2 distribution (SINGH 1949). The frequency distribution of group II could be calculated by adding both the distributions of group I and P_2, then dividing by two, since this group, i.e., group II, lies between both frequencies. This was based on the assumption that complete genic and phenotypic dominance exist. Table 6 gives the theoretical frequency distributions of the F_2 generation based on the above assumption.

TABLE 6

The F_2 generation frequency distribution partitioned into its component genotype on the basis of three-factor pairs for the number of days from seeding to silking

Population and genotype	Classes in percentages					Theoretical percentage
	56–59	60–63	64–67	68–71	72–75	
1. *AABBCC*	2.50	17.50	55.00	22.50	2.50	1.5625
2. *AABBCc*	2.01	45.52	49.15	3.33	.....	28.1250
3. *AABbCC*						
4. *AABbCc*						
5. *AaBBCc*						
6. *AaBbCC*						
7. *AaBBCC*						
8. *AaBbCc*	5.13	30.77	43.59	12.83	7.70	12.5000
9. *Aabbcc*	6.40	44.91	47.68	1.01	.....	28.1250
10. *AaBbcc*						
11. *Aabbcc*						
12. *aabbCc*						
13. *aaBbcc*						
14. *aaBbCc*						
15. *AaBBcc*	1.67	15.00	48.34	30.00	5.00	23.4375
16. *AAbbCC*						
17. *AAbbCc*						
18. *AABBcc*						
19. *AABbcc*						
20. *aaBBCC*						
21. *aaBBCc*						
22. *aaBbCC*						
23. *AabbCC*						
24. *aabbCC*	.84	12.50	41.67	37.50	7.50	4.6875
25. *aaBBcc*						
26. *AAbbcc*						
27. *aabbcc*	.....	10.00	35.00	45.00	10.00	1.5625
F_2 population	3.48	33.82	47.38	12.67	2.68	

The χ^2 test for goodness of fit between the obtained and the theoretical F_2 frequency distributions gave a χ^2 value of 5.2938 with a P value lying between 0.20 and 0.10 (Table 7), indicating a good fit and supporting the hypothesis that the two parents were differentiated by three major gene pairs with respect to silking date.

2. Pollen shedding

The mean values for the number of days from seeding to pollen shedding showed that P_1 (E.G. 102) and P_2 (E.G. 205) had mean values of 62.68 and 66.57 days respectively (Table 8).

The F_1 cross between the two parents gave a mean value of 62.20 days, which was somewhat lower than that of P_1, but the difference was insignificant. This fact indicated a complete phenotypic dominance of fewer days over more days. If this were true, then the B_1 to P_1 mean should not differ significantly from the means of either P_1 or F_1. This was found to be true with respect to F_1 *vs.* B_1 to P_1, but not with P_1 *vs.* B_1 to P_1. However, since B_1 to P_1 gave a mean value less than B_1 to P_2, then the conclusion would be justified as to the presence of complete

TABLE 7

Chi-square for goodness of fit between the obtained and the theoretical frequency distributions of the F_2 population for the number of days from seeding to silking based on the assumption that the parents were differentiated by three gene pairs

Population	Classes					Number of individuals
	56–59	60–63	64–67	68–71	72–75	
	In percentages					
Obtained	1.95	30.51	46.11	16.89	4.54	154
Calculated	3.48	33.82	47.38	12.67	2.68	154
	In numbers					
Obtained	3	47	71	26	7	154
Calculated	5.36	52.08	72.97	19.51	4.13	154
$\chi^2 = 5.2938$ P .20–.10	d.f. = 3 (the last two classes on the left were grouped in one)					

TABLE 8

The means, standard deviations, variances and number of individuals in each population for the number of days from seeding to pollen shedding

Population	$\bar{X} \pm$ S.D.	Total variance	Environmental variance	Genetic variance	Number of individuals
P_1	62.68 ± 2.08	3.4067	3.1679	.2388	38
B_1 to P_1	61.61 ± 2.05	3.6264	2.7932	.8332	126
F_1	62.20 ± 1.80	3.1142	2.9998	.1144	35
F_2	62.88 ± 2.53	6.2577	3.2380	3.0197	139
B_1 to P_2	63.18 ± 2.60	6.3635	3.3430	3.0205	132
P_2	66.57 ± 2.43	4.1771	4.5302	−.3531	46

phenotypic dominance. The justification was arrived at by comparing the frequency distributions of the three populations, namely P_1, F_1, and B_1 to P_1. χ^2 test gave a value of 1.6177 with a P value of .50–.30 (d.f. 2).

By studying both the total variances and the genetic variances for P_1, F_1 and B_1 to P_1, it can be seen that the total variances did not differ significantly from each other and were less than those of F_2 and B_1 to P_2. This supported the assumption of the occurrence of phenotypic dominance. By studying the genetic variances for the six populations it can be seen that the genetic variances of both F_2 and B_1 to P_2 were much higher than B_1 to P_1, which indicated complete genic dominance.

Number of major gene pairs differentiating parents: On the basis of the segregating generations, Table 9, it was suggested that the parents were differentiated by two major gene pairs. This was arrived at from the fact that dividing 18.19 percent (class 66–74 of B_1 to P_2) by 77.14 percent (class 61–65 of F_1) gave a value of 23.58 percent, whereas on a two-factor basis a ratio of 25 percent would be expected. Also the indication was arrived at from the fact that only 10.88 percent of the individuals in the F_2 population fell in the class 66–74, whereas 6.25 percent would be expected on the basis of two factors. When the theoretical means of F_2 and B_1 to P_2 for one factor gene pair, two and three pairs were compared with the obtained means of these two populations (Table 10), it appeared that the calculation based on the hypothesis that the two inbreds were differentiated by two major gene pairs was the fit.

TABLE 9

Condensed frequency distribution for the number of days from seeding to pollen shedding in percentage

Population	Classes in percentages			Number of individuals
	56–60	61–65	66–74	
P_1	21.05	78.95	.....	38
B_1 to P_1	30.16	69.84	.....	126
F_1	22.86	77.14	.....	35
F_2	20.83	68.28	10.88	139
B_1 to P_2	16.67	65.15	18.19	132
P_2	.....	34.78	65.22	46

TABLE 10

Theoretical values for the number of days from seeding to pollen shedding based on hypothesis that indicated the number of gene pairs differentiating the two inbreds

Number of gene pairs	Mean for	
	F_2	B_1 to P_2
1	63.65	64.63
2	62.91	63.53
3	62.80	63.17
Actual	62.88 ± 2.53	63.18 ± 2.60

Following this, the backcrosses were partitioned into their component genotypes, Table 11.

Following this partition, a theoretical frequency distribution could be calculated for the F_2 population, Table 12. The χ^2 test for goodness of fit between the theoretical and the obtained F_2 frequency distributions gave a χ^2 value of .5469 with a P value of .80–.70 (d.f. 2) indicating a good fit and supporting the hypothesis that the two parents were differentiated by two major gene pairs, Table 13.

3. *The difference in days between silking and pollen shedding*

From the mean values given in Table 14, it can be seen that inbred E.G. 102 (P_1) started shedding pollen 2.48 days, on the average, before silking. Mean-

TABLE 11

Partitioning of B_1 to P_1 and B_1 to P_2 frequency distributions into their component genotypes for the number of days from seeding to pollen shedding

Population and genotype	Row	Classes in percentages				Theoretical percentage
		56–60	61–65	66–70	71–74	
B_1 to P_1	1	30.16	69.84	.....	.....	100.00
$F_1 + P_1$ (*AaBb* + *AABB*)	2	10.98	39.03	.....	.....	50.00
Row 1 — row 2	3	19.18	30.81	.....	.....	50.00
Row 3 (100% basis)	4	38.37	61.63	.....	.....	100.00
F_1 (*AaBb*)	5	22.86	77.14	.....	.....	25.00
P_1 (*AABB*)	6	21.05	78.95	.....	.....	25.00
B_1 to P_2	7	16.67	65.15	16.67	1.52	100.00
$F_1 + P_2$ (*AaBb* + *aabb*)	8	5.72	27.99	14.68	1.63	50.00
Row 7 — row 8	9	10.95	37.16	1.99	—.11	50.00
Row 9 on a positive basis	10	10.95	37.16	1.88	.....	50.00
Row 10 (100% basis)	11	21.90	74.32	3.76	.....	100.00
F_1 (*AaBb*)	12	22.86	77.14	.....	.....	25.00
P_2 (*aabb*)	13	.....	34.78	58.70	6.52	25.00

TABLE 12

The F_2 frequency distribution partitioned into its component genotypes on the basis of two-factor pairs for the number of days from seeding to pollen shedding

Population and genotype	Classes in percentages				Theoretical percentage
	56–60	61–65	66–70	71–74	
AABB	21.05	78.95	.....	.....	6.25
AaBB	30.16	69.84	.....	.....	25.00
AABb					
AaBb	22.86	77.14	.....	.....	25.00
AAbb	16.67	65.15	16.67	1.52	12.50
aaBB					
Aabb	16.67	65.15	16.67	1.52	25.00
aaBb					
aabb	.....	34.78	58.70	6.52	6.25
F_2	20.83	68.28	9.91	.97	

while, in the inbred E.G. 205 (P_2), the differences were 1.50 days. The magnitude of the difference between the two parents was .98 days. This difference was shown to be highly significant.

The means of P_1, F_1 and B_1 to P_1 were approximately the same. These means indicated complete phenotypic dominance of longer duration between silking and pollen shedding. The total variances of the three populations, P_1, F_1, and B_1 to P_1 were approximately the same, indicating complete genic dominance.

Number of gene pairs differentiating the two parents: The assumption was made that the two parents were differentiated by one major gene pair. This assumption was reached from the fact that only 28.29 percent of the individuals in the F_2 population fell in the class 0–1, whereas, on a one gene pair, 25 percent would be expected (Table 15). The assumption was also arrived at by dividing 48.97 percent (class 2 or more of B_1 to P_2) by 76.46 percent (the same class for F_1) which gave a value of 64.05 percent. On the basis of one pair of genes, a 50 percent value would be expected.

On the assumption that one gene pair was responsible for the difference between silking and pollen shedding in the two parents, where P_1 was the dominant one, it would be expected that the frequency distributions of the three

TABLE 13

Chi-square test for goodness of fit between the obtained and theoretical F_2 frequency distributions for the number of days from seeding to pollen shedding based on the assumption that the two inbreds are differentiated by two gene pairs

Population	Classes				Number of individuals
	56–60	61–65	66–70	71–74	
	In percentages				
Obtained	26	96	17	.....	139
Calculated	20.83	68.28	19.91	.97	139
	In numbers				
Obtained	26	96	17	.....	139
Calculated	28.95	94.91	13.77	1.35	139

$\chi^2 = .5469$ d.f. $= 2$ P $= .80$–$.70$ (last two classes on left were grouped in one)

TABLE 14

The means, standard deviations, the variances and the number of individuals in each population for the difference in days between silking and pollen shedding

Population	$\bar{X} \pm$ S.D.	Total variance	Environmental variance	Genetic variance	Number of individuals
P_1	2.48 ± 1.12	1.2707	1.1598	.1109	33
B_1 to P_1	2.46 ± 1.25	1.4349	1.1628	.2721	113
F_1	2.41 ± 1.13	1.3450	1.1704	.1746	34
F_2	2.55 ± 1.58	2.5258	1.1492	1.3766	152
B_1 to P_2	1.54 ± 1.25	1.4522	1.3020	.1502	145
P_2	1.50 ± 1.06	1.0226	1.3081	—.2855	36

TABLE 15

Condensed frequency distribution, in percentage, for the differences in days between silking and pollen shedding

Population	Classes in percentages		Number of individuals
	0–1	2-more	
P_1	9.09	90.90	33
B_1 to P_1	21.24	78.75	113
F_1	23.52	76.46	34
F_2	28.29	71.71	152
B_1 to P_2	51.03	48.97	145
P_2	52.78	47.22	36

populations, P_1, F_1, and B_1 to P_1, would be the same or differ within the limits of random sampling. This was found to be the case, since χ^2 value was 2.8532 which gave a P value of .30–.20 (d.f. 2). χ^2 tests for goodness of fit for the ratio 3:1 in the F_2 population gave a P value of .50–.30. Also the χ^2 test for the ratio 1:1 in the B_1 to P_2 population gave a P value of .90–.80. All these supported the hypothesis that the difference between the two parents was governed by a major gene pair.

DISCUSSION AND CONCLUSION

The development of an organ or an organism involves an organized system of interrelated and interdependent physiological processes, the end result of which is the manifestation of the phenotypic character. In this system the effects of most genes are integral parts (WRIGHT 1934). In such studies it was necessary to recognize both phenotypic and genic dominance. Phenotypic dominance was determined by studying the means of the two parents and the mean of the F_1 generation. This relation could be checked by studying the mean of the backcross to the dominant parent. Genic dominance was determined from studying the means, variances and phenotypes of the different genotypes. However, it should be realized that dominance is a relative phenomenon and its manifestation differs according to the genic background of the different gene combinations (FISHER 1931; WRIGHT 1934; DOBZHANSKY and HOLZ 1943).

"Fewer" days from seeding to silking and to pollen shedding showed complete phenotypic dominance over "more" days. The genetic variance indicated also complete genic dominance. SINGH (1949) found complete phenotypic and genic dominance for fewer days in the squash from seeding to opening of the first male and the first female flower. The same thing was reported by POWERS (1941) in tomatoes. It was found from these studies that silking date was determined by at least three major gene pairs by which the two inbred lines were differentiated. The number of days from seeding to pollen shedding was found to be governed by at least two major gene pairs. The difference in days between pollen shedding and silking indicated complete phenotypic and genic dominance for longer duration with at least one major gene pair controlling the difference.

The author, in 1954, by using chromosomal interchanges in maize, reported at

least two gene pairs responsible for the number of days from seeding to silking and at least three gene pairs for the number of days from seeding to pollen shedding. He concluded that since his observations were made on the same plants, and since only two of the genes indicated by the associations in pollen shedding studies were in the same arm as those for silking date, it appeared that not all the genes controlling pollen shedding and silking dates were the same.

Later the author, in 1956, in his studies on the effect of environment on gene segregation, using the same material used in 1954, reported that at least two major gene pairs controlled silking date and that one major gene pair controlled pollen shedding date under Alexandria, Egypt, conditions. The author concluded that the gene controlling pollen shedding date could be the same as that controlling silking date but the other gene which was found to be present in silking date could act as a differential one.

In the present studies, it can be stated that in the early stages of development, two major gene pairs would be responsible for pollen shedding and silking dates. However, a third gene probably interacts with the other two genes on the female side, i.e., silking date, and causes the silk appearance to be delayed more than that of pollen shedding. This conclusion can support that reported earlier by the author, i.e., the two characters are not the same but some genes are common to both. This can by no means be considered conclusive until more linkage data are available.

This finding should be of considerable importance to maize breeders who are breeding for earliness. If we consider what the author reported in 1954 and 1956, we could assume that the three genes are independent. Therefore, the breeder can combine earliness in his varieties since it is somewhat simply inherited. This earliness is essential in the northern parts of Egypt where the corn borer is abundant in August. Thus, earlier varieties will have a better chance to escape the damage done by this pest.

SUMMARY

1. The maize inbred line (E.G. 102) was crossed to the inbred (E.G. 205) raised by the Ministry of Agriculture, Egypt, in its program for producing hybrid corn.

2. By using the partitioning method suggested by POWERS (1942) and POWERS *et al.* (1950) it was possible to estimate the number of gene pairs controlling the number of days from seeding to silking and pollen shedding.

3. The number of days from seeding to silking and the number of days from seeding to pollen shedding were differentiated by three and two major gene pairs, respectively.

4. The differences in days between silking and pollen shedding was found to be governed by one major gene pair.

5. There was complete phenotypic and genic dominance for fewer days from seeding to silking and from seeding to pollen shedding, as well as for the longer duration of the difference in days between silking and pollen shedding.

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TRANSPOSITIONS OF MODULATOR, A COMPONENT OF THE VARIEGATED PERICARP ALLELE IN MAIZE[1]

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GENES, in the classical sense of the term, have fixed chromosome positions that can be determined by linkage tests with other genes. These units change position in relation to the genes on either side only as a result of chromosomal rearrangements. McCLINTOCK (1951) discovered genetic elements in maize that do not regularly occupy the same position in the genome but are capable of undergoing transposition from one locus to another by some mechanism other than ordinary chromosomal rearrangement. Modulator (*Mp*) an element associated with variegated pericarp and cob in maize (P^{vv} in EMERSON's terminology, 1917) is such a transposable unit. *Mp* is similar in action to, or identical with, a factor which McCLINTOCK termed Activator (*Ac*).

Modulator was interpreted by BRINK and NILAN (1952) as a factor which, when present at the *P* locus in conjunction with the P^{rr} gene (self-red pericarp, red cob) suppresses pigmentation. P^{vv}, therefore, may be equated to $\overline{P^{rr}Mp}$. Removal of *Mp* from the $\overline{P^{rr}Mp}$ complex results in full color expression of P^{rr}. The ear phenotype termed medium variegated, which comprises numerous red stripes of various sizes on a colorless background, was considered to be the result of sporadic losses of *Mp* from the *P* locus in somatic tissue in plants heterozygous for variegated and a colorless pericarp allele. Progeny tests show that another, less conspicuous but about equally frequent, mutation called light variegated accompanies the mutations to self-color. Light variegated pericarp plants were found to differ from medium variegateds in possessing an additional Modulator (transposed Modulator= *tr-Mp*) at some position in the genome other than the *P* locus. This genotype may be designated, therefore, as $\overline{P^{rr}Mp}/P^{wwv} + tr\text{-}Mp/\text{-}$. Evidence from twinned red and light variegated sectors on medium variegated ears, and from other sources, points to the conclusion that the second Modulator (*tr-Mp*) present in light variegated arises by transposition of *Mp* to one or another site when variegated mutates to self-red at the *P* locus. Transposed Modulator reduces the frequency of *Mp* losses from the *P* locus in $\overline{P^{rr}Mp}$ plants about 60 percent (BRINK 1954), thus resulting in the light variegated phenotype.

The present study deals with the distribution of the sites to which Modulator is transposed when the element is removed from the *P* locus in the mutation of

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$\overline{P^{rr}Mp}$ to P^{rr}. The criterion used to identify plants carrying *tr-Mp* was the capacity of this element to change the variegated phenotype from medium to light.

MATERIALS AND METHODS

The variegated pericarp allele used was derived originally from a single plant heterozygous for colorless pericarp and cob. It was introduced into a few locally adapted inbred dent strains, at least three backcrosses to the respective recurrent parent lines having been made before the present tests were begun.

A series of independently occurring mutations from medium to light variegated were assembled in these inbred strains by propagating sublines from (1) single light variegated ears in otherwise medium variegated families (2) light variegated sectors on medium variegated ears (3) light variegated sectors that were twinned with self-red mutations, and (4) single-kernel light variegated mutants on medium variegated ears. Each of the light variegated mutants thus isolated (heterozygous for the variegated pericarp allele and hemizygous for transposed Modulator) was mated at once with a colorless pericarp inbred strain. Progenies were then grown out, and the open-pollinated ears were harvested and classified for pericarp color. This direct testing procedure reduced to a minimum the opportunity for secondary transpositions of *tr-Mp* to occur; and the uniformity of genetic background, incident to the use of inbred parental lines, facilitated identification in the testcross families of the two main classes of offspring, light and medium variegated plants.

As noted earlier, plants heterozygous for colorless pericarp and the variegated allele are medium variegated, whereas addition to this genotype of transposed Modulator in single dose changes the phenotype to light variegated. If *tr-Mp* is located at a position not linked with *P*, then by random assortment of *tr-Mp* and *P* half the variegated plants resulting from testcrossing such light variegated heterozygotes will be light and half will be medium. If *tr-Mp* is linked with *P*, then more than half the variegated segregates will be light, the higher the percentage of individuals in this class, the closer the linkage indicated.

Preliminary to the above mentioned study a series of light variegated mutants, that had appeared in our cultures earlier and had been propagated through two or more additional generations, were crossed with stocks carrying reciprocal translocations marking most of the 20 chromosome arms. These matings were made with a view to determining the distribution of transposed Modulators, wherever the latter were situated in the genome. Two facts soon came to light that led to a change in this rather unwieldy experimental plan. It was found that, after propagation of a given light variegated mutant through a generation or so, position in the genome of transposed *Mp* often varied from one subline to another, presumably as the result of secondary transpositions. Furthermore, in a rather high proportion of the independently occurring mutants it was observed that *tr-Mp* was present on chromosome 1, linked with *P*. Attention was then redirected to newly arisen mutations of medium to light variegated and to the determination

among these mutations of the relation of *tr-Mp* to the *P* locus only, as outlined earlier in this section of the report.

Validation of the method used for estimating linkage of transposed Modulator with the P *locus*

The method employed in estimating the amount of recombination between transposed Modulator and the *P* locus in the present investigation was applicable to the variegated pericarp segregates only, following testcrosses with colorless pericarp of light variegated plants heterozygous for colorless pericarp and hemizygous for transposed Modulator. The light variegated phenotype ($\overline{P^{rr}Mp}/P^{wiwi}$ + *tr-Mp*/-) served as the criterion for the presence of transposed Modulator in contrast to medium variegated ($\overline{P^{rr}Mp}/P^{wiwi}$) in which *Mp* is present in the genome as a component of the variegated allele only. Very light variegated plants (occurring as mutants with an over-all frequency of roughly three percent of all colored segregates) were included in the light variegated class on the grounds that they usually arise from the latter genotype and differ only in possessing an additional *Mp* element (BRINK 1954). Mutations of variegated to self-red pericarp, which occur with a similar low frequency, were not scorable for the presence or absence of *tr-Mp* by the method in question, and so were omitted from the computations. This procedure, it will be noted, leaves out of consideration all the colorless pericarp individuals, comprising one half the testcross populations. The question may properly be asked whether a valid estimate of the amount of recombination between *tr-Mp* and the *P* locus can be obtained from the variegated segregates only.

Conceivably, linkage between *tr-Mp* and the variegated pericarp allele, is spurious in part as a result of the two *Mp* units present in the nuclei of light variegated plants tending to pass to the same pole at the heterotypic division in meiosis regardless of the location of *tr-Mp* in the genome. That such preferential assortment does not occur was demonstrated by the following experiment.

Testcross families representing 23 independently occurring mutations from medium to light variegated were reared under conditions in which the colorless pericarp individuals, as well as the variegateds, could be scored for presence and absence of *tr-Mp*. If the two *Mp* units involved do not assort preferentially then estimates of the distribution of *tr-Mp* within the respective colored and colorless classes should correspond to each other.

The 23 light variegated mutants, heterozygous for colorless pericarp, and hemizygous for transposed Modulator ($\overline{P^{rr}Mp}/P^{wiwi}$ + *tr-Mp*/-) were mated with a colorless pericarp (P^{wiwi}) inbred strain not carrying *Mp*. These stocks were homozygous for colorless aleurone (*cc*). The testcross offspring were reared in an isolation plot and, following detasseling, were pollinated by a strain carrying Dissociation (*Ds*) at the standard position on chromosome 9 (MCCLINTOCK 1953) and also the dominant aleurone color factor, *C*, in a distal location on the same chromosome arm. The detasseled plants were scored at maturity both for pericarp color and the presence or absence of *C* to *c* mottling in the aleurone. The

C to *c* mottling occurs as the result of losses to the *C Ds/c ds/c ds* aleurone nuclei of the chromosome fragment bearing the dominant *C* marker, by virtue of breakage at the *Ds* locus, thus giving rise to lineages of colorless cells. Such breakage takes place only when both *Mp* and *Ds* are present in the nucleus. Thus the *C* to *c* mottling provides a specific test for occurrence of *Mp* (presumably at any locus) in the genotype. The question at issue is the extent to which the distribution of *tr-Mp* in the colorless pericarp class of segregates, as thus determined, corresponds with the proportion of light and medium variegateds among the colored offspring.

The relevant data are summarized in Table 1. Inspection of the numerical results shows that (1) the amount of recombination indicated by both criteria varies widely from family to family in this group and (2) estimates of this value based upon the proportion of colorless offspring with and without *tr-Mp* correspond reasonably well in each case to that which would have been obtained had the numbers of light and medium variegated segregates alone been recorded. The regular correspondence between the distribution of *tr-Mp* between the colored and colorless pericarp classes within families shows that any tendency for the two *Mp* units present in the light variegated genotype to assort together in meiosis can be no more than a minor factor conditioning the proportion of medium and light variegated segregates if, indeed, it is operative at all.

RESULTS

Preliminary tests for distribution of tr-Mp *throughout the genome*

The 350 testcross families scored for light and medium variegated and for one or another of a series of reciprocal translocations marking most of the 20 chromosome arms showed that *tr-Mp* can be found in a variety of positions in the genome both linked and nonlinked to the *P* locus. It was apparent from these data also that, in terms of total length of the chromosome complement, *tr-Mp* was linked with *P* on chromosome 1 in a disproportionately large number of cases. Linkage was definitely established in two cases to reciprocal translocations not involving chromosome 1. In one of these cases *tr-Mp* showed about 34 percent recombination with T5-9a, and in the other about 30 percent recombination was found with T4-5b. Data from another group of families showing linkage between *P*, *tr-Mp*, and one or another reciprocal translocation involving chromosome 1 suggested that *tr-Mp* was located on the respective complementary chromosome involved in the exchange. The translocations giving such evidence were T1-3a, T1-4b, T1-2d, and T1-5b. Thus evidence was obtained that *tr-Mp* can occupy positions on chromosomes 2, 3, 4, 5, and 9, as well as various positions on chromosome 1.

Tests of newly arisen mutations to light variegated

The results of testing for recombination between *P* and *tr-Mp* of new mutations to light variegated are summarized in Table 2. The groups of mutations originally

TABLE 1

Distribution of transposed Modulator among colorless pericarp offspring in relation to proportion of light and medium variegated segregates following testcrosses with colorless of light variegated plants heterozygous for colorless and hemizygous for tr-Mp

	Number of plants					
	Colored pericarp			Colorless pericarp		
				tr-Mp		
Family	Light var.	Medium var.	Percent medium	present	absent	Percent present
73– 1	27	29	51.8	28	40	41.2
73– 2	31	30	49.2	27	31	46.6
73– 3	54	44	44.9	36	62	36.7
73– 4	32	7	17.9	7	26	21.2
73– 5	17	20	54.1	20	13	60.6
73– 6	72	4	5.3	3	64	4.5
73– 7	39	12	23.5	13	35	27.1
73– 8	38	2	5.0	0	34	0
73– 9	48	36	42.9	38	49	43.7
73–10	28	19	40.4	19	32	46.3
73–11	54	0	0	1	51	1.9
73–12	54	0	0	5	62	7.2
73–13	91	1	1.1	3	92	3.2
73–14	24	1	4.0	3	15	16.7
73–15	13	23	63.9	11	17	39.3
73–16	25	19	43.2	20	21	48.8
73–17	17	19	52.8	28	22	56.0
73–18	28	29	50.9	22	29	43.1
73–19	46	10	17.9	13	45	22.4
73–20	21	12	36.4	21	22	48.8
73–21	36	39	52.0	29	38	43.3
73–22	58	3	4.9	4	46	8.0
73–23	44	2	4.3	3	60	4.8

detected as twinned with a red pericarp sector, single kernels, or entire light variegated ears appeared not to show different recombination values, and so are considered together. Of the 87 families tested, each representing an independent mutation to light variegated, 56 showed linkage of *P* and *Mp*. The distribution of these 56 cases, in terms of recombination value with *P*, is shown in Figure 1. It is evident that there is a progressive increase in frequency as the position of *tr-Mp* approaches the *P* locus, that is, as the percentage of medium variegated plants declines. Distribution of *tr-Mp* on the two sides of *P* was not determined in these experiments. There is also a high occurrence (25 of 87 families, or 29 percent) of cases showing no recombination between *P* and *tr-Mp*. Twenty-nine of the 87 mutants yielded percentages of medium variegated segregates that did not differ significantly ($P>.05$) from 50 percent.

If at the time of the differential mitosis in which *Mp* is liberated from the *P* locus, *Mp* moved at random to a new position in the chromosome complement, only a position within 50 crossover units on either side of the *P* locus that carried the unchanged $P^{rr}M\bar{p}$ gene would show linkage with *P*. Taking into consideration

TABLE 2

Percentage of medium variegated segregates among the testcross offspring of independent, newly arisen light variegateds

Family number	No. var. ears	Percent med. var.	Family number	No. var. ears	Percent med. var.
6D– 58	59	40.7*	N–35	9	0
63–514	94	2.1	–37	80	10.0
–519	71	19.7	–38	25	4.0
–523	125	34.4‡	–40	25	48.0*
72–621	65	12.3	–42	48	56.2*
–625	60	51.7*	–44	67	0
–630	45	13.3	–47	25	0
–631	67	1.5	–49	24	0
–632	67	0	–53	35	8.6
–633	192	7.2	–59	33	42.4*
–635	162	19.1	–61	48	0
–636	70	61.1*	–65	25	0
–637	61	49.2*	–66	33	42.4*
–638	28	49.9*	–70	36	0
–639	30	53.3*	–71	37	2.7
–642	85	70.6‡	–75	30	0
–643	70	31.4‡	–83	29	0
–645	78	67.9‡	–85	19	0
–650	95	52.6*	–89	20	45.0*
–652	95	42.1*	73–1	56	51.8*
–659	70	48.6*	–2	61	49.2*
–1160	33	54.4*	–3	98	44.9*
–1161	34	0	–4	39	17.9
–1164	28	3.6	–5	37	54.1*
–1167	15	0	–6	76	5.3
–1170	40	0	–7	51	23.5
–1172	30	13.3	–8	40	5.0
–1173	46	2.2	–9	84	42.9*
–1175	25	0	–10	47	40.4*
–1179	17	0	–11	54	0
–1183	22	0	–12	54	0
–1189	20	50.0*	–13	92	1.1
–1190	39	0	–14	25	4.0
N–4	49	0	–15	36	63.9*
–6	31	6.5	–16	44	43.2*
–9	79	57.0*	–17	36	52.8*
–10	52	5.8	–18	57	50.9*
–12	50	2.0	–19	56	17.9
–13	16	25.0	–20	33	36.4*
–16	35	51.4*	–21	75	52.0*
–27	32	0	–22	61	4.9
–28	33	0	–23	46	4.3
–30	13	7.7	–24	35	0
–32	25	0			

* Not significantly different from 50 percent ($P > .05$).
‡ Differs very significantly from 50 percent ($P < .01$).

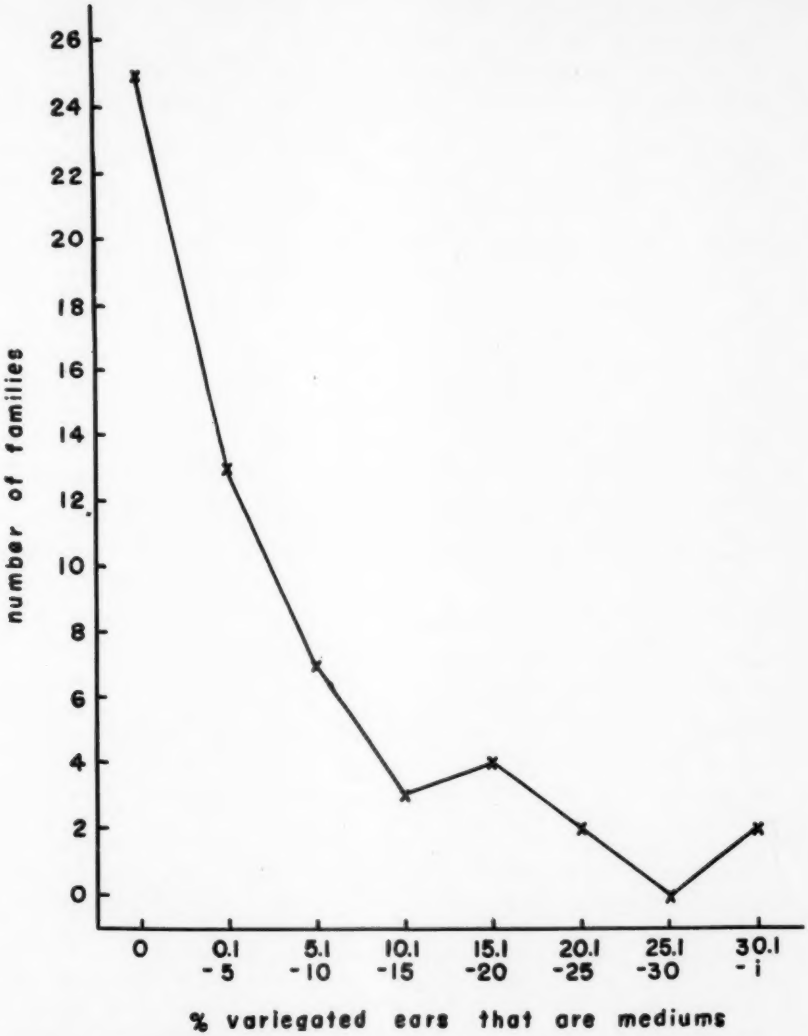

FIGURE 1.—Amount of recombination between the *P* locus and transposed Modulator, as measured by the percentage of medium variegated segregates in testcross families, for 56 independently occurring mutants to light variegated.

TABLE 3

Pedigrees showing varying degrees of stability of tr-Mp *position*

Pedigree			Family no.	Total var.	Percent meds.
6–13–1	→72–143–2	→72–319–2	→72–816	53	35.8
79% lts:	7 lts:	–3	→72–817	36	69.4†
	2 med.–4	→72–318–1	→72–814	34	17.6
		–2	→72–815	47	57.4*
		→72–321–1	→72–818	46	6.5
		–6	→72–819	29	10.3
		→72–324–2	→72–825	44	45.4*
		→72–327–2	→72–828	47	29.8
	–7	→72–317–1	→72–813	55	20.0
		→72–325–1	→72–826	25	20.0
	–8	→72–322–1	→72–820	44	36.4
		–5	→72–821	61	14.7
	–11	→72–323–1	→72–823	56	3.6
		–3	→72–822	65	0
		–6	→72–824	55	3.6
		→72–326–1	→72–827	47	4.3
		→72–328–1	→72–829	51	3.9
		–2	→72–830	64	3.1
			Total	859	
6–184–14	→72–147–1	→72–355–1	→72–855	34	0
19 lts:	23 lts:	–3	→72–856	42	0
1 med.	1 med.	→72–358–3	→72–858	65	3.1
		→72–366–1	→72–863	47	2.1
		→72–377–2	→72–874	37	0
	–8	→72–357–4	→72–857	58	6.9
		→72–359–3	→72–859	35	2.8
	–18	→72–370–2	→72–867	43	4.6
		–3	→72–868	44	0
	–28	→72–365–1	→72–862	18	0
	–29	→72–374–1	→72–873	63	1.6
	–30	→72–363–2	→72–860	39	10.3
		–6	→72–861	18	0
	–32	→72–373–3	→72–872	45	0
	–37	→72–369–2	→72–866	64	4.7
	–42	→72–368–2	→72–865	43	2.3
	–54	→72–371–1	→72–869	119	4.2
		–2	→72–870	30	0
		–3	→72–871	53	5.7
	–56	→72–367–4	→72–864	51	2.0
	–64	→72–376–1	→72–875	17	0
		–3	→72–876	25	0
			Total	981	

6–196–101	→6–245		→6–304	→72–651	91	0
12 lts. only						
102	→6–262–1			→72–540	120	0
	15 lts–2			→72–541	146	0
	only –3			→72–542	118	0
		–4	→Bk 952	→72–432	46	0
			→72–414–1	→72–683	164	0
			–2	→72–684	111	0
			–3	→72–685	104	0
				Total	900	

* Not significantly different from 50 percent ($P > .05$).
† Differs significantly from 50 percent ($P < .05$).

the differing lengths of the maize chromosomes, it was estimated that 100 crossover units is equal to approximately 52.4 micra. This was computed by averaging the micra per 100 crossover units for chromosomes one and two, which are the longest and most uniformly marked, as given by RHOADES (1950). The total length at pachytene of the ten maize chromosomes is 552 micra. There are, therefore, an estimated 1050 crossover units on the ten chromosomes. This figure is probably a minimum. Only at a position within 100 of these 1050 units does *tr-Mp* show linkage with the *P* locus. If *tr-Mp* transposes from the *P* locus at random, only in a maximum of 9.5 percent of cases would *tr-Mp* be expected to show linkage with *P*. Of the 87 independent positions listed in Table 2, however. 56 or 64 percent show linkage with *P*.

Secondary transpositions

If the new position assumed by Modulator at the time of its primary transposition from the *P* locus were stable, then all sister families, that is. light variegated families arising from progeny of kernels from the same mutant sector on the foundation ear, should have *tr-Mp* at the same position. The pedigrees given in Table 3 show that this is not always the case. Sister families often show quite different percentages of medium variegated ears, suggesting that *tr-Mp* may continue to move after its initial transposition. Some lineages give evidence of frequent changes of *tr-Mp* position, while in others the position seems to be stable over a number of generations. Examples of these two types of pedigrees are given in Table 3. Only variegated plants, and not the colorless pericarp segregates, are shown in the pedigrees. The first pedigree given, that showing descendants of plant 6-13-1. shows positions of *tr-Mp* ranging from complete linkage with *P* to independence. At least four distinct positions linked with *P* are indicated: one between three and four units from *P*, one approximately 15 units from *P*, one about 20 units from *P*, and another about 35 units from *P*. In the second pedigree all the values could be estimates of the same position of *tr-Mp*. The third pedigree given is one in which all descendants of the sister plants 6-196-101 and -102 show no recombination between *P* and *Mp*. Pedigrees such as these suggest that there may be differences in the stability of *tr-Mp* in different positions. The examination of several such pedigrees suggested that this stability may vary according to the degree of linkage between *tr-Mp* and the variegated pericarp allele.

TABLE 4

Comparisons of linkage between variegated pericarp and tr-Mp *in descendants of plants that showed various amounts of linkage between variegated pericarp and* tr-Mp

Parental family		Daughter family		Parental family		Daughter families	
Percent meds.	Var. ears	Var. ears	Percent meds.	Percent meds.	Var. ears	Var. ears	Percent meds.
0	36	40	2.5	7.1	28	71	1.4
		31	0			62	3.2
		54	0				
		47	0	8.6	70	60	6.7
		23	0				
				9.7	103	54	48.1*
0	120	146	0			51	47.1*
		118	0				
		46	0	13.9	72	38	7.9
		164	0			24	4.2
		111	0				
		104	0	16.7	36	98	3.1
0	93	85	0	19.6	92	56	23.2
		59	0				
				20.0	10	63	33.3
0	46	95	5.6				
		18	4.5	27.2	136	9	44.4*
						29	37.9*
0	51	43	4.7			23	56.5*
		32	3.1			12	58.3*
0	27	38	5.3	27.6	62	35	60.0*
		83	0				
				30.4	23	26	53.8*
0	37	64	0				
		44	2.3	34.6	26	84	14.3
0	67	29	0	39.3	112	38	34.2
		25	8.0			20	45.0*
		14	14.3			21	47.6*
1.0	101	47	4.3	40.8*	49	32	37.5*
		35	8.6				
		80	2.5	42.3*	104	28	57.1*
						39	51.3*
1.0	102	34	0				
				45.7*	46	26	3.8
1.7	119	42	4.8			34	2.9
		60	0				
		40	0	52.5*	40	18	27.8*
1.9	54	43	0	53.3*	45	21	57.1*
2.7	37	43	2.3	57.4*	104	28	57.1*
		27	0			39	51.3*
3.1	64	23	4.3	58.9*	56	109	56.9*
		27	0			14	21.4†
		14	14.3				
		27	0	60.9*	23	43	55.8*
3.6	83	55	1.8	62.5*	40	67	52.2*
		40	2.5				
6.4	47	36	0				

* Not significantly different from 50 percent ($P > .05$).
† Differs significantly from 50 percent ($P < .05$).

There were a few cases among the families grown during the course of this study in which seed from a single testcross ear was grown for classification of the progeny and also some daughter ears were testcrossed again, and the resulting progeny then grown for classification. In these groups of related families it is possible to test for successive transpositions of *Mp*. Such families are shown in Table 4, arranged according to the percent of recombination between *P* and *tr-Mp* in the stem family. In the stem families which indicated complete linkage or up to five percent recombination between *P* and *tr-Mp*, none of the daughter families show recombination values very different from that of the parent. Among the descendants of stem plants in which there was 5.1 percent to 10 percent recombination between *P* and *tr-Mp*, there seems to have been at least one move that resulted in a change from a recombination value of 9.7 percent in the parent to independence with *P* in the two daughter families. In the families in which the parent showed 10 percent to 25 percent crossing over between *P* and *tr-Mp*, moves have apparently taken place in the first family, in which the parent gave 13.9 percent recombination, as compared to 7.9 percent and 4.2 percent for the two daughter families, and in the second family in which the parent gave 16.7 percent recombinations as compared to 3.1 percent for the daughter family.

In stem families showing recombination values of 25 percent or greater (but omitting cases not significantly different from independent assortment, $P>.05$) there is evidence for at least four different moves of *tr-Mp*: at least one in the first pedigree where daughter families gave independent assortment while the parent gave only 27.2 percent crossing over; one in the second pedigree where the parent family gave 27.6 percent and the daughter showed independent assortment; one in the third pedigree—30.4 percent compared to independent assortment, and one in the fourth, 34.6 percent compared to 14.3 percent. Among the offspring of families in which *P* and *tr-Mp* assorted independently in the stem family, at least three cases are in evidence in which *tr-Mp* subsequently became linked with the *P* locus.

Moves from one position to another outside of 50 crossover units on either side of *P*, of course, cannot be detected. Short moves and moves from one side of *P* to about the same distance on the other side were not revealed by the technique used, so the estimates of the numbers of moves given in Table 4 are minimum values. Chi-square tests for independence were used where possible, but because of small plant numbers these tests were not always informative. The decisions as to when moves are indicated, therefore, are somewhat arbitrary. The results of Table 4 can be summarized as follows:

Percent crossovers *P-Mp* in parent	Total daughter families	*tr-Mp* moves indicated	Percent of moves
0	24	0	0
0.1–5	16	0	0
5.1–10	6	2	33

10 –25	5	2	40
25 up	10	4	40
indep.	13	3	23

These data suggest that *tr-Mp*'s located close to the *P* locus are less likely to move in successive generations than are *tr-Mp*'s located farther from the *P* locus. Because of the small numbers of families available for the test, however, such a relationship cannot be proved conclusively.

DISCUSSION AND CONCLUSIONS

The present data show that the sites in the genome that Modulator may occupy vary widely, thus corroborating a conclusion which McCLINTOCK (1951) had previously reached with reference to elements of this kind on the basis of her studies with Activator and Dissociation.

The criterion here used for the detection of *tr-Mp* was the modifying effect of the element on variegated pericarp. It is significant that the modifying action, as observed, is essentially the same regardless of *tr-Mp* position in the genome (cf. KEDHARNATH and BRINK 1958). The fact that variable or graded phenotypic effects are not in evidence suggests that *tr-Mp* in variegated pericarp plants does not become subliminal in any position. By the same token, the sample of *tr-Mp* positions disclosed by the technique used may be considered unbiassed.

Of the 87 independently occurring transpositions tested, nearly two thirds were to sites linked with *P*, the initial position of Modulator on the short arm of chromosome 1. A computation based on total length at pachytene of all ten maize chromosomes and on the linkage map shows that if *Mp* transposed to other loci at random, then no more than one tenth of the new sites would be linked with *P*. There is a strong preference in transposition, therefore, for new sites of *Mp* in the vicinity of the old one.

The data establish the additional fact that the sites on chromosome 1 to which *Mp* is transposed are ordered in terms of recombination with *P*. The likelihood of occupation by *tr-Mp* of a particular linked site is found to be a function of the proximity of that site to *P*.

Some evidence was obtained suggesting that if transposed Modulator occupies a position close to *P* it is less likely to undergo secondary transposition than if it is located elsewhere on chromosome 1. This point requires further study. McCLINTOCK (1956b) has shown that *Ds*, which ordinarily is readily transposable (provided *Ac* also is present in the genome) may become stable in position. BRINK (1958) has described a case in which Modulator similarly became fixed at the *P* locus. There is no clue regarding the basis of such differences in transposability.

One looks in vain in the present data for evidence that the transpositions of Modulator are related to gene activation and deactivation in ways that are meaningful for development of the organism. As a component of the variegated pericarp allele, *Mp* suppresses the pigment-producing action of the P^{rr} gene. The latter, when present at the *P* locus alone, gives self-red pericarp and cob. Although

the point has not been established directly in the case of *Mp*, it may be presumed that the element conditions gene expression similarly at the various other loci to which it transposes. These transposition sites are diverse. The majority of them, however, lie in the same chromosome, in which they tend to be distributed in declining frequency as the crossover distance between the original *Mp* locus and the new position increases. There is no evidence that the genes over this long region are similarly ordered in terms of developmental action. One concludes, therefore, that the movements of *Mp* from one chromosome position to another are not of a kind that would be expected to impose a functional order on the gene system.

The present findings, on the other hand, suggest that mechanical factors play a predominant, although probably not an exclusive, role in the transposition of Modulator from one chromosome site to another. Further progress in the analysis of *Mp* mobility is dependent on gaining some knowledge of the processes whereby *Mp* is released from one position, transported to a new site, and then reincorporated in the chromosome.

SUMMARY

Of 87 independently occurring transpositions of Modulator (*Mp*), a component of the variegated pericarp allele at the *P* locus on chromosome 1 in maize, to other chromosome sites, 56 were found to be to positions linked with *P*. Furthermore, the closer the new site to *P*, the more likely was it to be occupied by a transposed Modulator. Evidence was obtained that Modulator also transposes to chromosomes 2, 3, 4, 5 and 9. Following a primary transposition of *Mp* from the *P* locus, the frequency of secondary transpositions varies widely from case to case.

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A FURTHER STUDY ON INDUCTION OF FUNCTIONAL SEX REVERSAL IN GENOTYPIC MALES OF THE MEDAKA (ORYZIAS LATIPES) AND PROGENIES OF SEX REVERSALS[1, 2]

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DESPITE a great deal of work on artificial control of gonadal sex differentiation in every class of vertebrates, functional sex reversal induced by administration of sex hormones has rarely been achieved. Complete and functional sex reversal in genetic males under the influence of estrogens (either estrone or stilbestrol) was successful in a cyprinodont fish, the medaka (*Oryzias latipes*). Induced sex reversals of genetic males in mating with normal males produced viable offspring (YAMAMOTO 1953). GALLIEN (1954a,b) demonstrated that functional sex reversal in genetic males of the newt (*Pleurodeles waltlii*) can be induced by estradiol benzoate. GALLIEN (1955, 1956) and CHANG and WITSCHI (1955) succeeded in inducing functional sex reversal in genetic males of the clawed toad (*Xenopus laevis*) by treatment with estradiol. In both the newt and the clawed toad, progenies of sex reversals by normal males were only males, indicating that the female is heterogametic (WZ) and the male homogametic (ZZ). In amphibians and higher vertebrates, however, functional sex reversal from female to male by administration of male sex hormone had not heretofore been achieved. In the medaka, induction of functional sex reversal in genetic females under the influence of methyl testosterone was also successfully accomplished (YAMAMOTO 1958).

In an oviparous cyprinodont, the medaka (*Oryzias latipes*) in which the sex-determining mechanism is XX for female and XY for male, either the gene *R* (orange-red) or *r* (white) is borne by the X or Y chromosomes (AIDA 1921). When a white female (X^rX^r) is mated with a heterozygous orange-red male (X^rY^R), the progeny are white females (X^rX^r) and orange-red males (X^rY^R) in equal numbers. In matings of this sort, white (*r*) fish are females and orange-red (*R*) are males because the gene *R* for a dominant orange-red condition is located only in the Y chromosome. Hence the *r* and *R* can be used as markers of sex genotypes XX and XY.

The father-to-son inheritance is always displayed except in rare exceptional fish in which the *R* gene dissociates itself from the sex-limited inheritance. Since the

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R and *r* are partially sex-linked, exceptions such as X^RX^r females or X^rY^r males can be produced by crossing over between the heterosomes. Exceptions such as X^rX^r males or X^rY^R females may arise by genic imbalance between the sex chromosomes and autosomal sex genes. The total of the exceptions in our stock (d-*rR* strain) is less than one percent. Hence, in our breed, the *r* and *R* can be used as markers of sex genotypes XX and XY with a reliability of more than 99 percent.

It has already been shown that by using offspring of these matings XY individuals can be inverted in their gonadal sex differentiation and that they will develop into complete and functional females by continuous administration *per os* of estrogens (estrone and stilbestrol) for several months after hatching (YAMAMOTO 1953). Induced sex reversals of male genotype (X^rY^R) were mated with normal orange-red males (X^rY^R). A number of the F_1 orange-red sons were individually mated with separate white females (X^rX^r) in tests for either X^rY^R or Y^RY^R sons. A single F_1 orange-red son among the estrone-sex-reversed females was actually identified as the very Y^RY^R male which had fathered all 72 male offspring. Induction of sex reversal of the male genotype (XY) by means of estrone was thus conclusively demonstrated by the occurrence of a YY male among F_1 offspring of sex reversals (YAMAMOTO 1955).

The present paper is a continuation of the study of the induction of reversal of gonadal sex differentiation in genetic males (XY) made under the influence of the estrogens, and of the progenies of sex reversals. While in this further experiment, the dosage level of estrone is the same as that used in the previous study (1,250 i.u./g diet), the higher level of stilbestrol in the previous study was lowered in this study to the same level of international unit as estrone. The period of administration for both estrogens in two of the three experimental series of this study was limited to the time between hatching and certain juvenile stages. This was done to ascertain the period for hormone administration sufficient to insure reversal of sex differentiation. Our success in inducing reversal of sex differentiation through use of estrogens led to the conviction that sex reversal of Y^RY^R zygotes would be induced by the same means. To realize this, sex reversal in genotypic males must be induced in two consecutive generations. Such a purpose was carried out in the present study.

MATERIALS AND METHODS

A genetically analyzed breed (d-*rR* strain) of the medaka (*Oryzias latipes*) was used as material. Parents were products of mass breeding of heterozygous orange-red males (X^rY^R) and white females (X^rX^r). Before time of breeding, a stock census was conducted and all the detected rare exceptions were excluded.

Since the present experiments were run on two or more generations, where the first one or two generations were estrogenized, the following symbols are used to denote the offspring.

N : Normal-fed fish of one generation. White female is N*r* ♀ and orange-red male N*R* ♂.

E : Estrone-fed fish of one generation. Estronized white female is E*r* ♀ and estrone-sex-reversed orange-red female E*R* ♀.

S : Stilbestrol-fed fish of one generation. Stilbestrolized white female is S*r* ♀ and stilbestrol sex-reversed orange-red female S*R*♀.

N*r*♀ >N : Normal-fed offspring produced by mating normal-fed white females (X^rX^r) with normal orange-red males (X^rY^R). White daughter is N*r*♀ >N*r*♀ and orange-red son N*r*♀ >N*R*♂.

E*r*♀ >N : Normal-fed offspring produced by mating estronized white females (X^rX^r) with normal orange-red males (X^rY^R). White daughter is E*r*♀ > N*r*♀ and orange-red son E*r*♀ >N*R*♂.

E*R*♀ >N : Normal-fed offspring produced by mating estrone-sex-reversed orange-red females (X^rY^R) with normal orange-red males (X^rY^R). White daughter is E*R*♀ >N*r*♀ and orange-red son E*R*♀ >N*R*♂.

E*R*♀ >E : Estrone-fed offspring produced by mating estrone-sex-reversed females (X^rY^R) with normal orange-red males (X^rY^R). Estronized white daughter is E*R*♀ >E*r*♀ and sex-reversed orange-red daughter E*R*♀ >E*R*♀.

S*r*♀ >N : Normal-fed offspring produced by mating stilbestrolized white females (X^rX^r) with normal orange-red males (X^rY^R). White daughter is S*r*♀ >N*r*♀ and orange-red son S*r*♀ >N*R*♂.

S*R*♀ >N : Normal-fed offspring produced by mating stilbestrol-sex-reversed females (X^rY^R) with normal orange-red males (X^rY^R). White daughter is S*R*♀ >N*r*♀ and orange-red son S*R*♀ >N*R*♂.

S*R*♀ >S : Stilbestrol-fed offspring produced by mating stilbestrol-sex-reversed females (X^rY^R) with normal orange-red males (X^rY^R). Stilbestrolized white daughter is S*R*♀ >S*r*♀ and stilbestrol-sex-reversed daughter S*R*♀ >S*R*♀.

Other symbols designating exceptions are given in proper places. To assist the reader to visualize experiments, diagrams are introduced into the text (Figures 3 and 4).

Normal diet of fry and juveniles consists of 60 g shrimp powder, 30 g toasted whole barley flour, 6 g yeast preparation and 4 g powdered green tea. As estrogen diet, tablets of commercial preparation were finely ground in a mortar and homogeneously mixed with the normal diet. The dosage level of both estrone and stilbestrol is 1,250 i.u. (estrone 125 μg and stilbestrol 62.5 μg) per gram diet.

Two series of experiments for induction of sex reversal were performed. In one, estrogens were administered to fish *per os* for about seven months after hatching, as in the previous study. Newly hatched fry average about 4.8 mm in total length, varying from 4.5 to 5.4 mm. By the end of the administration period, fish had reached 14–24 mm. It may be remarked that fish are mature when they reach about 28 mm. In the other series of experiments, estrogens were administered to the first generation for 4–6 weeks, and to the second for more than six weeks from the time of hatching, until young reached 8–13 mm and 10–15 mm, respectively. Definite developmental stages were taken as the time for discontinuation of hormone treatment instead of using definite periods of estrogen administration because fish exhibit great variability in growth rate. Fry were reared indoors

under windows in three-liter glass jars during the administration of estrogen diet. Upon reaching either of these definite stages mentioned above, young fish were successively reared outdoors in concrete pools (75 cm square and 18 cm deep) until they reached sexual maturity. The method employed encourages large numbers of full-grown sex reversals because rearing of fish out-of-doors greatly reduces mortality. The young fish under outdoor conditions were fed on the mixture of shrimp powder and toasted whole barley flour with the addition occasionally of live tubificids and water fleas.

The total length measuring from the snout to the end of the caudal fin is given for size of fish. Unless otherwise stated, the sex referred to is the sex phenotype instead of the sex genotype.

RESULTS

1. *Effect of estrogen feeding from hatching up to stage of 14–24 mm and results of testcrosses of estrogenized fish:* Between July 1st and 15th, the daily hatchings were divided into three groups; the first was fed normal diet (N), the second estrone diet (E) and the third stilbestrol diet (S). Thus the total hatchings for fifteen days were of three diet classifications and these diets were successively administered for several months. By the end of this period, fish had reached the stage of 14–24 mm. Thereafter they were reared outdoors until they reached sexual maturity. The experimental result is presented in the upper section of Table 1. Whatever the sex genotype, all the estrogenized fish differentiated into females. It follows that all the estrogen-administered orange-red fish (X^rY^R) had inverted their genetically determined sex and differentiated into females, while control orange-red fish had developed into normal males. Both estrogenized white females and orange-red sex reversals were found to be fertile. Sex reversals of the male genotype (X^rY^R) not only have body configuration and secondary sex characters similar to those of normal females, but they have feminine sex equip-

TABLE 1

Sex reversal in genotypic males (X^rY^R) *of the medaka induced by administration of estrogens for seven months (14–24 mm) and progenies of sex reversals of male genotype and estrogenized genotypic females*

Symbol of offspring	Parents ♀	Parents ♂	Diet of offspring	Period of estrogen feeding	Offspring r♀	Offspring r♂	Offspring R♀	Offspring R♂
N	X^rX^r	X^rY^R	Normal	.....	5	0	0	8
E	X^rX^r	X^rY^R	Estrone	H→14–24 mm	5	0	7	0
S	X^rX^r	X^rY^R	Stilbestrol	H→14–24 mm	14	0	9	0
Nr♀>N	X^rX^r	X^rY^R	Normal	.....	9	0	1	15
Er♀>N	X^rX^r	X^rY^R	Normal	.....	106	0	3	120
ER♀>N	X^rY^R	X^rY^R	Normal	.....	18	0	1	32
Sr♀>N	X^rX^r	X^rY^R	Normal	.....	53	0	0	55
SR♀>N	X^rY^R	X^rY^R	Normal	.....	11	0	0	28

Dosage level of estrogens = 1,250 i.u./g diet. r = White. R = Orange-red, H = Hatching time, N = Normal-fed, E = Estrone fed, S = Stilbestrol fed.

ment and capacity. Evidently XY zygotes had developed into functional females under the influence of estrogens. An estrone-sex-reversal female (*ER* ♀, X^rY^R) and a stilbestrol-sex-reversal female (*SR* ♀, X^rY^R) are illustrated in Figures 1 and 2, respectively.

Estrogenized white females (X^rX^r) and sex-reversed orange-red females (X^rY^R) were mated with normal orange-red males (X^rY^R). The result is shown in the lower half of Table 1. The estrogenized genotypic females (X^rX^r), either estronized white fish (*Er* ♀) or stilbestrolized white ones (*Sr* ♀), in mating with normal orange-red males (X^rY^R) produced white daughters and orange-red sons in approximately 1:1 ratio as is the case of control white females (*Nr* ♀). Four estrone-sex-reversal *ER* ♀ ♀ (X^rY^R) and nine stilbestrol-sex-reversal *SR* ♀ ♀ (X^rY^R) in mating with two and five normal males (X^rY^R), respectively, produced offspring in which orange-red sons preponderate (2–3 times) over white daughters. In the offspring denoted by *ER* ♀ >N, the ratio of white daughters to orange-red sons is close to 1:2(χ^2=0.16, P=0.70–0.50) rather than that of expected 1:3(χ^2=3.2, P=0.10–0.05). This is ascribed to the rarity of Y^RY^R males.

2. *Effect of estrogen-feeding from hatching to 8–13 mm stage:* In another series of experiments, either estrone or stilbestrol was administered to fry from hatching to juvenile stage of 8–13 mm. Thereafter they were reared on normal diet until they became fully grown. The result is shown in the upper section of Table 2. The controls (N) had developed into white females and orange-red males in equal numbers, as expected. Out of 57 estronized fish (E), 26 were white females (*Er* ♀ ♀) and 31 were orange-red fish of which 28 were females (*ER*

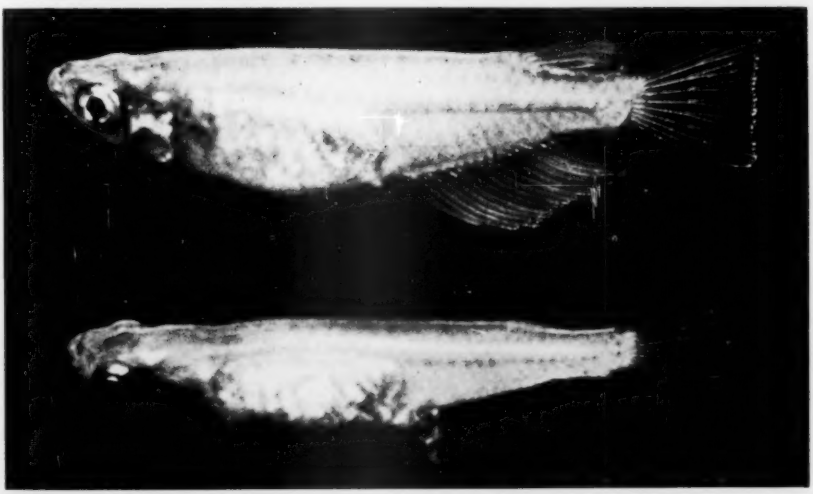

FIGURE 1.—(Above) Control orange-red male (*NR* ♂, X^rY^R); (Below) Functional estrone-sex-reversal (*ER* ♀) of male genotype (X^rY^R) carrying a cluster of fertilized eggs. × 3.2.

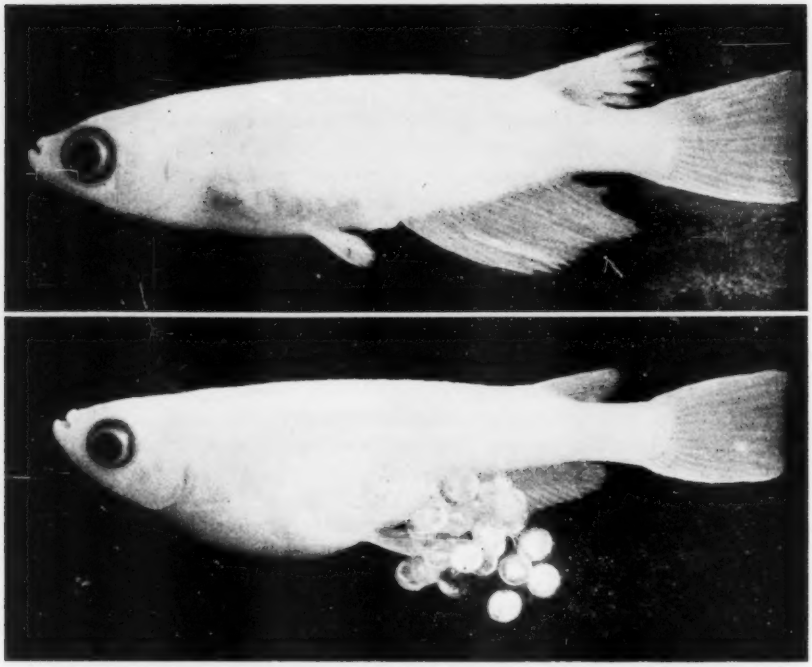

FIGURE 2.—(Above) Control orange-red male ($NR\delta$, X^rY^R); (Below) Functional stilbestrol-sex-reversal ($SR\varphi$) of male genotype (X^rY^R) carrying a cluster of fertilized eggs. × 3.2.

TABLE 2

Sex reversal in genotypic males of the medaka induced by estrogens in one and two consecutive generations

Symbol of offspring	Parents ♀	Parents ♂	Diet of offspring	Period of estrogen feeding	Offspring r♀	Offspring r♂	Offspring R♀	Offspring R(i.s.)	Offspring R♂
N	X^rX^r	X^rY^R	Normal	----------	24	0	0	0	22
E	X^rX^r	X^rY^R	Estrone	H→ 8–13 mm	26	0	28	1	2
S	X^rX^r	X^rY^R	Stilbestrol	H→ 8–13 mm	49	0	67	0	0
Er♀ >N	X^rX^r	X^rY^R	Normal	----------	45	0	0	0	55
ER♀ >N	X^rY^R	X^rY^R	Normal	----------	16	1	0	0	52
ER♀ >E	X^rY^R	X^rY^R	Estrone	H→10–15 mm	7	0	24	0	1
Sr♀ >N	X^rX^r	X^rY^R	Normal	----------	6	0	0	0	5
SR♀ >N	X^rY^R	X^rY^R	Normal	----------	34	2	3	0	86
SR♀ >S	X^rY^R	X^rY^R	Stilbestrol	H→10–15 mm	4	0	5	0	0

The period of administration covers from hatching to certain juvenile stages, as noted above. Dosage level of estrogens = 1,250 i.u./g diet. r = White, R = Orange-red, H = Hatching time, N = Normal fed, E = Estrone-fed, S = Stilbestrol fed.

♀♀), one androintersex and two were males ($ER♂♂$). Obviously 90 percent of genotypic males (X^rY^R) had reversed their sex differentiation and developed into phenotypic females. The single intersex was characterized by having secondary sex characters of both sexes in intermediate state as well as having a hermaphroditic gonad. An account has already been given elsewhere of the gonad of this intersex (YAMAMOTO 1957).

Apparently, regardless of the sex genotype, all the stilbestrolized fish (S) had developed into females. Evidently 100 percent of genotypic males (n=67) had differentiated into females under the effect of stilbestrol at the dosage level of 1,250 i.u./g diet. This dosage is one fourth that used in the previous study. At the same international unit, stilbestrol is evidently more effective than estrone. When we compare the effectiveness of stilbestrol and estrone in weight per gram diet, the potency of the former is more than twice that of the latter, since 1 i.u. of estrone is 0.1 μg while that of stilbestrol is 0.05 μg.

3. *Progenies of estrogen-treated fish to 8–13 mm and induction of sex-reversal in two consecutive generations:* It should be noted that not only do estrogen-sex-reversals have structural traits appropriate to normal females, but their sexual behavior is the same. Mass matings in separate groups were performed on estronized genetic females (15 Er♀♀) and estrone-sex-reversals (9 ER♀♀) of the male genotype (X^rY^R) with normal orange-red males (X^rY^R). Of the stilbestrolized fish, stilbestrolized genotypic females (25 Sr♀♀) and stilbestrol-sex-reversals (43 SR♀♀) of the male genotype (X^rY^R) were mated with normal orange-red males (X^rY^R) in two separate groups. Most of the estrogenized fish were found to be fertile. Offspring of either the estronized white females (Er♀♀) or stilbestrolized white females (Sr♀♀) were reared on normal diet.

Daily hatchings (July 2–8) of two groups, *viz.*, one of estrone-sex-reversals (ER♀♀) mated with normal males and the other of stilbestrol-sex-reversals (SR♀♀) with normal males, were used. The first group was divided, one half reared on normal diet designated as ER♀>N (*cf.* Figure 3 and Table 2) and one half on estrone diet denoted as ER♀>E (*cf.* Figure 4 and Table 2). The second group was divided in the same way, one half reared on normal diet designated as SR♀>N and one half on stilbestrol diet denoted as SR♀>S. In both groups, the half fed on normal diet (ER♀>N and SR♀>N) served as controls for fish estrogenized in two generations (ER♀>E and SR♀>S). Fry in the other half of the two groups were reared on their respective hormone diets until reaching 10–15 mm stage. The normally fed offspring (Er♀>N) of estronized genotypic females (Er♀♀) mated with normal orange-red males (X^rY^R) were white daughters and orange-red sons in equal numbers. The same was true of normally fed offspring (Sr♀>N) produced by stilbestrolized genotypic females (Sr♀♀) and normal orange-red males. The normally fed offspring (ER♀>N) of estrone-sex-reversal females (ER♀♀, X^rY^R) mated with normal orange-red males (X^rY^R) (*cf.* Figure 3), were 16 white daughters and 52 orange-red sons with one exceptional white son (lower section of Table 2). The ratio of white daughters to orange-red sons approximates the expected 1:3. The number in this case is, how-

ever, too small to give much significance to agreement with the theoretical expectancy. The normally fed offspring (S*R* ♀ >N) produced by stilbestrol-sex-reversals (S*R* ♀ ♀, X^rY^R) and normal orange-red males (X^rY^R) comprised 34 white daughters, 86 orange-red sons with two exceptional white males and three

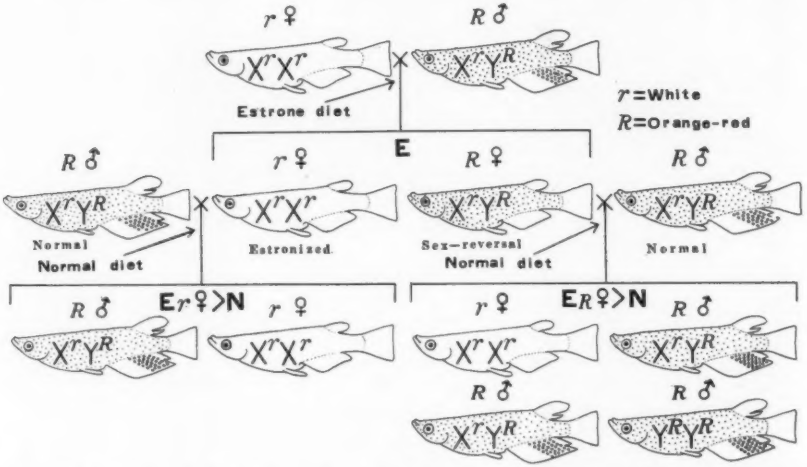

FIGURE 3.—Diagram of induction of sex reversal in genotypic males (X^rY^R) of the medaka by estrone in one generation and normal-fed progenies of sex reversals and of estronized genotypic females (X^rX^r).

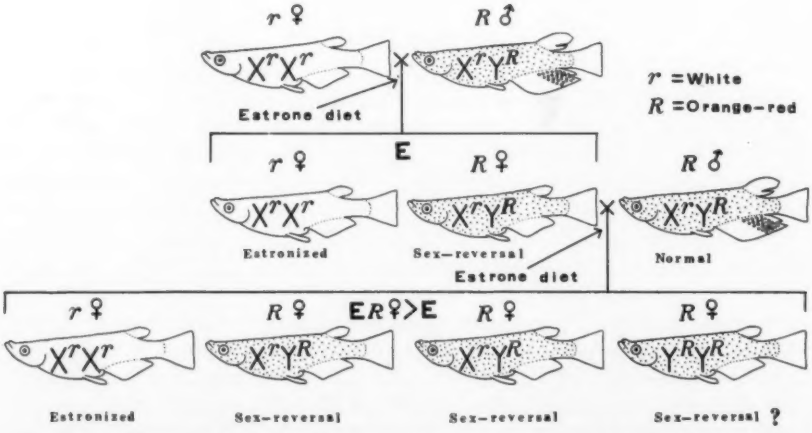

FIGURE 4.—Diagram of induction of sex reversal in genotypic males of the medaka by estrone in two consecutive generations.

exceptional orange-red females. The ratio of white daughters to orange-red sons was 1:2.5 instead of the theoretical 1:3.

Estronized offspring ($ER♀ > E$) produced by mating estrone-sex-reversals ($ER♀♀$, X^rY^R) with normal orange-red males (X^rY^R) (*cf.* Figure 4) were seven white daughters and 24 orange-red daughters with one orange-red son. Obviously 24 out of 25 (96 percent) genotypic males (X^rY^R and Y^RY^R) had inverted their sex differentiation and developed into females, with only one genotypic male remaining nonreversed.

Stilbestrolized offspring ($SR♀ > S$) of stilbestrol-sex-reversals ($SR♀♀$, X^rY^R) mated with normal orange-red males (X^rY^R) comprised four white daughters and five orange-red daughters. Evidently under the influence of stilbestrol all the genotypic males (X^rY^R and Y^RY^R) had reversed their sex and developed into functional females.

4. *Testcrosses of F_1 orange-red sons of sex-reversals estrogenized for one generation:* Normally fed F_1 orange-red sons, which had been produced by matings of estrogen-sex-reversals (X^rY^R) estrogenized to 8–13 mm stages with normal orange-red males (X^rY^R), were submitted to genetic analysis. Theoretically, we could expect that there would be two kinds of males, the X^rY^R and Y^RY^R, among normally fed F_1 orange-red sons (*cf.* Figure 3). Because Y^RY^R males cannot be distinguished from the X^rY^R males except by testcrosses, a random selection was made of ten out of 52 normal-fed orange-red sons ($ER♀ > NR♂$) of estrone-sex-reversals and six normal-fed orange-red F_1 sons ($SR♀ > NR♂$) of stilbestrol-sex-reversals. These males were singly tested by white females (X^rX^r). Results showing fully grown offspring from 16 matings are given in the upper section of Table 3. The results of all matings that yielded both sexes in equality are consolidated in Table 3. Of ten F_1 orange-red sons ($ER♀ > NR♂$) of estrone-sex-reversals tested by white females (X^rX^r), nine produced offspring consisting of white females and orange-red males in approximately equal numbers, a total

TABLE 3

Testcrosses of F_1 males of estrogen-sex-reversals in one generation (upper section) and of fish estrogenized in two consecutive generations (lower section)

Parents ♀	Parents ♂	No. of matings	Offspring $r♀$	Offspring $r♂$	Offspring $R♀$	Offspring $R♂$	Genotype of fish tested
X^rX^r	$ER♀ > NR♂$ a	9	841	5	10	911	X^rY^R
X^rX^r	$ER♀ > NR♂$ b	1	0	0	1	354	Y^RY^R
X^rX^r	$SR♀ > NR♂$	6	594	1	4	711	X^rY^R
$ER♀ > ER♀$	X^rY^R	16	496	7	35	1,012	X^rY^R
X^rX^r	$ER♀ > ER♂$ *	1	51	0	0	56	X^rY^R
$ER♀ > Er♀$	X^rY^R	5	115	0	2	100	X^rX^r
$SR♀ > SR♀$	X^rY^R	5	55	2	3	106	X^rY^R
$SR♀ > Sr♀$	X^rY^R	1	23	0	0	22	X^rX^r

* $ER♀ > ER♂$ on the second line in lower section is the single nonreversed orange-red son of estrone-sex-reversed mother (cf. Table 2). r = White, R = Orange-red, N = Normal fed. E = Estrone fed, S = Stilbestrol fed.

of 841 r♀♀ and 911 R♂♂ with five exceptional r♂♂ and ten exceptional R♀♀. Obviously the nine parents tested (ER♀>NR♂a) bred as X^rY^R males. Only one (ER♀>NR♂b) of the ten tested proved to be a Y^RY^R male and this extraordinary fish is seen in Figure 5. He fathered a total of 355 orange-red offspring of which 354 were males and one was an exceptional female. One of these 354 orange-red sons (ER♀>NR♂b>NR♂) presumably having the genotype (X^rY^R), in mating with white females (X^rX^r) actually sired 20 white females and 24 orange-red males, proving his constitution to be X^rY^R. The single exceptional orange-red daughter (ER♀>NR♂b>N*exc.R*♀) in two matings with X^rY^R males, produced 30 white females and 92 orange-red males (or 1r♀:3R♂) with one exceptional orange-red female, as shown in the third line in the upper section of Table 4.

TABLE 4

Testcrosses of exceptions among F_2 offspring of estrogen-sex-reversals in one generation (upper section) and of exceptions among estrogen-sex-reversals in two consecutive generations (lower section)

Parents ♀	Parents ♂	No. of matings	Offspring r♀	Offspring r♂	Offspring R♀	Offspring R♂	Genotype of *exc.* fish
X^rX^r	ER♀>NR♂a>N*exc.r*♂	3	88	96	0	0	X^rY^r
ER♀>NR♂a>N*exc.R*♀	X^rY^R	1	5	0	0	21	X^rY^R
ER♀>NR♂b>N*exc.R*♀	X^rY^R	1	30	0	1	92	X^rY^R
SR♀>NR♂>N*exc.R*♀	X^rY^R	1	2	0	0	8	X^rY^R
X^rX^r	ER♀>ER♀>N*exc.r*♂	1	9	11	0	0	X^rY^r
ER♀>ER♀>N*exc.R*♀	X^rY^R	2	72	0	70	86	X^rX^R
X^rX^r	SR♀>SR♀>N*exc.r*♂	2	71	58	0	0	X^rY^r
SR♀>SR♀>N*exc.R*♀	X^rY^R	1	3	0	0	8	X^rY^R

r = White, R = Orange-red, N = Normal-fed, E = Estrone fed, S = Stilbestrol fed, *exc.* = Exceptional.

FIGURE 5.—The second Y^RY^R male detected among normal-fed F_1 orange-red sons (ER♀>NR♂) of estrone-sex-reversal X^rY^R females mated with normal X^rY^R males. This Y^RY^R male in mating with white females (X^rX^r) fathered 354 orange-red sons and a single orange-red daughter. × 3.2.

Obviously the single daughter of the Y^RY^R male was of the constitution X^rY^R. This exception might have arisen by genic imbalance between heterosomes and autosomal sex genes.

The single Y^RY^R male in the present study is the second Y^RY^R male to have been detected among F_1 offspring of estrogen-sex-reversal females mated with normal males. When the first Y^RY^R male, previously reported (YAMAMOTO 1955), was mated with white females (X^rX^r), all 72 offspring were orange-red males. The first Y^RY^R male was found to be exceedingly virile in secondary sex characters. The papillar processes on the anal fin are the most prominent feature among the male-positive sex characters. The second Y^RY^R male, 32 mm when sacrificed, had a total of 310 papillar processes. This number is high as compared with the average of those of ordinary XY males of the same size.

All six normal-fed F_1 sons ($SR♀ > NR♂$) of stilbestrol-sex-reversals ($SR♀$, X^rY^R) by normal X^rY^R males were proved to be X^rY^R by the fact that each of them in mating with white females (X^rX^r) fathered white females and orange-red males in nearly equality. The consolidated data are given on the third line in upper section of Table 3. The lack of Y^RY^R males among fish ($SR♀ > NR♂$) tested is probably due to the inadequate number of individuals in these tests.

5. *Progenies of fish estrogenized for two consecutive generations:* Fish estrogenized for two consecutive generations were subjected to testcrosses. The results are presented in the lower section of Table 3. Twenty-four estrone-sex-reversals ($ER♀ > ER♀$, *cf.* lower half of Table 2) produced by sex-reversal in two consecutive generations (*cf.* Figure 4) were singly tested by normal orange-red males (X^rY^R) for the purpose of deciding the genotype of sex-reversals for either X^rY^R or Y^RY^R. Twenty-one out of 24 $ER♀ > ER♀$ were found to be fertile, five of which died after having produced a few fry which were not reared to adulthood. The other 16 estrone-sex-reversals produced offspring in sufficient numbers to decide their genotype. Since each of 16 matings yielded offspring comprising the same phenotypic classes and having the same ratios, all their offspring are listed together on the first line in the lower section of Table 3. Out of a total of 1,550 offspring, 496 white females, 1,012 orange-red males, seven exceptional white males and 35 exceptional orange-red females were produced. Obviously all 16 estrone-sex reversals in two consecutive generations had the constitution X^rY^R. No estrone-sex-reversal females of the constitution Y^RY^R were detected. One of the 16 estrone-sex-reversals which had been estrogenized in two consecutive generations and bred as X^rY^R is illustrated in Figure 6. When X^rY^R females are mated with X^rY^R males, we can expect that offspring will consist of white females (X^rX^r), ordinary orange-red males (X^rY^R) and extraordinary orange-red males (Y^RY^R) in a ratio of 1:2:1. The observed ratio of white daughters to orange-red sons was 1:2(χ^2=0.13, P=0.80–0.70) instead of the theoretical 1:3(χ^2=50, P<0.01). The discrepancy may be attributable to the rarity of Y^RY^R males.

A single estronized orange-red male ($ER♀ > ER♂$, nonreversal, *cf.* lower section of Table 2) was tested to X^rX^r females and bred as X^rY^R instead of Y^RY^R (second line in lower section of Table 3).

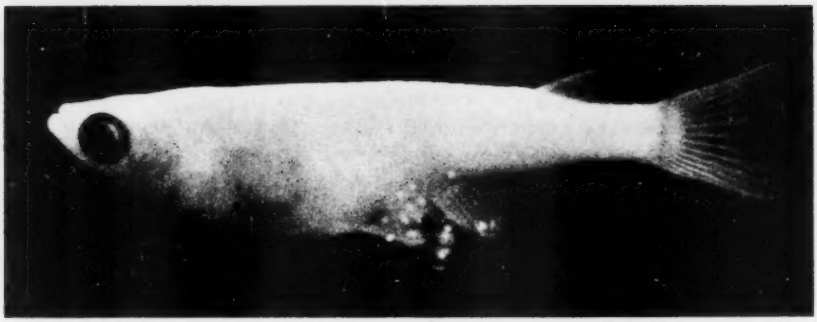

FIGURE 6.—Functional sex-reversal female (*ER*♀>*ER*♀) of male genotype treated with estrone in two consecutive generations. The genotype was proved to be X^rY^R. Note a cluster of fertilized eggs. × 3.2.

Five out of seven estronized white females (*ER*♀>*Er*♀, X^rX^r, *cf.* lower section of Table 2) were individually tested by normal orange-red males (X^rY^R). The results from all five matings are consolidated and listed on the third line in lower section of Table 3. The total offspring comprised 115 white daughters and 100 orange-red sons with two exceptional orange-red females.

Five stilbestrol-sex-reversals (*SR*♀>*SR*♀, *cf.* lower half of Table 2) produced by sex reversal in two consecutive generations and presumed to have either genotype X^rY^R or Y^RY^R, were singly tested to normal X^rY^R males. Each of the five matings gave white daughters and orange-red sons indicating that all these sex-reversal females had the genotype X^rY^R. The consolidated data are presented on the fourth line in the lower section of Table 3. Two exceptional white sons and three exceptional orange-red daughters were produced in addition to the expected phenotypic classes. In this case, also, the ratio of white daughters to orange-red sons is 1:2 ($\chi^2=0.05$, P=0.90–0.80) instead of the expected 1:3 ($\chi^2=7.2$, P<0.01). No Y^RY^R females were detected among females symbolized as *SR*♀>*SR*♀. Three out of four stilbestrolized white females *SR*♀>*Sr*♀ (X^rX^r), which were stilbestrolized daughters of stilbestrol-sex-reversal females, were mated to a normal X^rY^R male. As is shown on the fifth column in lower section of Table 3, the offspring comprised white daughters and orange-red sons in a 1:1 ratio, as expected.

6. *Testcrosses of exceptions:* Some exceptions produced among offspring of sex reversals in one and two consecutive generations were subjected to testcrosses. Exceptional white males were singly tested to two or three white X^rX^r females and exceptional orange-red females were individually tested to a normal X^rY^R male. The offspring from 12 successful matings are tabulated in Table 4. A separate record was kept on offspring of each mating. Results of matings yielding offspring in similar proportions of phenotypic classes are consolidated on the same line in Table 4. The progeny of an exceptional orange-red female (*ER*♀>*NR*♂b>*Nexc.R*♀) have already been described. All three exceptional white males (*ER*♀>*NR*♂a>*Nexc.r*♂) which are exceptions among F_2 offspring of

estrone-sex-reversals in one generation, were found to be X^rY^r arising from crossing over. One exceptional white male (*ER*♀ >*ER*♀ >N*exc.r*♂) and two exceptional white males (*SR*♀ >*SR*♀ >N*exc.r*♂), the exceptions among offspring of estrogen-sex-reversals in two consecutive generations, also were proved to be crossovers having the constitution X^rY^r.

One exceptional orange-red female (*ER*♀ >N*R*♂a>N*exc.R*♀) and one exceptional orange-red female (*SR*♀ >N*R*♂ >N*exc.R*♀), among F_2 offspring of estrone and stilbestrol sex reversals in one generation, tested by separate matings with X^rY^R males, produced progenies in which orange-red sons predominated over white daughters. One exceptional orange-red female (*SR*♀ >*SR*♀ > N*exc.R*♀) showed the same genetic behavior which proved her constitution to be X^rY^R.

Two exceptional orange-red females (*ER*♀ >*ER*♀ >N*exc.R*♀), the exceptions among offspring of estrone-sex-reversals in two consecutive generations, however, displayed quite a different genetic behavior. In matings singly with normal orange-red males X^rY^R, these two exceptional females produced offspring which comprised white daughters, orange-red daughters and orange-red sons. Consolidated data resulting from the two matings show 72 white daughters, 70 orange-red daughters and 86 orange-red sons, a ratio approaching a 1:1:1 instead of the expected 1:1:2. The sex distribution (142♀♀ and 86♂♂) is close to a ratio of 2♀:1♂ ($\chi^2=1.97$, $P=0.20$–0.10) rather than that of the expected 1♀:1♂ ($\chi^2=13.8$, $P<0.01$). Although the observed proportion showed a significant departure from the expected, we can not label these two exceptions other than X^rX^R, i.e., crossovers. A plausible explanation of the discrepancy is given in the eighth paragraph under Discussion. Since only two out of 35 exceptional orange-red females (*ER*♀ >*ER*♀ >N*exc.R*♀, *cf.* Table 4) were successfully tested, there is the possibility that X^rY^R females arising from genic imbalance might also be included among nontested fish.

Genetic analyses of exceptions showed that all the exceptional white males had the constitution X^rY^r arising from crossing over between the X^r and Y^R. However, exceptional orange-red females proved to have either the constitution X^rX^R resulting from crossing over or X^rY^R produced by genic imbalance between sex chromosomes and autosomal sex genes.

If exceptions were the result of reciprocal crossing over only, then the number of exceptional white males and orange-red females would be equal. The preponderance of the latter over the former is due to the occurrence of exceptional X^rY^R females in addition to crossover X^rX^R females. Exceptional X^rY^R females might be produced by an accumulation of the feminine genes in autosomes caused by recombination, as was maintained by WINGE (1934) for the guppy (*Lebistes reticulatus*).

DISCUSSION

According to the prevailing view the genetic sex determination is primarily dependent upon the male and female sex genes. The genic balance theory of sex

determination originally proposed by BRIDGES (1922, 1925, 1939) and later modified by WINGE (1934) has merit in explaining normal sex determination in the present fish. Although the sex genes are effective sex determiners, their action appears to be mediated by the sex gene controlled sex inductors (androinductor and gynoinductor). It has not yet been decided whether the steroid hormones are identical to, or allied with natural sex inductors, or if they are entirely different. The problem remains to be answered. In amphibians, WITSCHI (1942, 1955) stresses that natural sex inductors are not steroid hormones and are of protein nature. CHANG (1953), CHANG and WITSCHI (1956) and GALLIEN (1958) support the nonsteroid theory.

Our previous and present studies on the medaka indicate that an effective heterotypic sex hormone administered during larval life overrides the genetic mechanism for sex determination, *viz.*, the genetic male (XY) develops into functional female by estrogen and *vice versa* the genetic female (XX) differentiates into functional male by androgen, methyl testosterone (YAMAMOTO 1958). It can be stated that estrogen has an action of gynoinductor and methyl testosterone can act as an androinductor.

As shown in a previous paper (YAMAMOTO 1953), day-old fry (average length 4.8 mm) of the medaka have an indifferent gonad and their sex differentiation takes place during the larval period. The present study indicates that continuous administration of the estrogens at the dosage level of 1,250 i.u./g diet, beginning with hatching and ending at certain juvenile stages (8–13 or 10–15 mm), *i.e.*, covering the period prior to and passing through the stage of gonadal sex differentiation, is sufficient to induce reversal of sex differentiation in the majority of genetic males and to develop them into functional females. Continuation of administration after 12 mm seems to be superfluous. The result suggests that normally the sex genes play an active role only during a short period preceding the stage of gonadal sex differentiation. Perhaps, the sex genes become active and control the production of natural sex inductors within inductor cells surrounding indifferent gonia. These natural sex inductors may cause indifferent gonia to cyto-differentiate into either male or female germ cells.

An effective estrogen, administered during the proper period, may counteract and override the action of the male sex genes or that of the androinductor. Once the indifferent gonad in genetic males has undergone differentiation into ovary under the influence of an administered estrogen, the remaining sex equipment follows the female pattern under the control of endogenous ovarian activity which prevails after hormone administration has been discontinued. The sexual habits are those of the female, also. It is to be noted that the induction of sex reversal is permanent. There is no instance of sex-reversed fish which later turns into the genetically determined sex. On the other hand, we have had a number of sex reversals which produced offspring in two consecutive years.

In a previous study (YAMAMOTO 1955), a single Y^RY^R male was detected among 20 F_1 sons of estrone-sex-reversals and none among 21 F_1 sons of stilbestrol-sex-reversals. In the present study only one out of ten F_1 sons of estrone-sex-reversals

and none out of six F_1 sons of stilbestrol-sex-reversals was proved to be Y^RY^R male. When all these data are combined, it follows that only two Y^RY^R out of 57 F_1 sons (X^rY^R ♂ ♂ + Y^RY^R ♂ ♂) of sex reversals (X^rY^R) mated to X^rY^R males, *viz.*, approximately one Y^RY^R out of every 30 sons are viable instead of the theoretical one out of every three. This shows that only one out of every ten Y^RY^R zygotes can be viable. A lethal action of some sort seems to be operative in the majority of zygotes having the constitution Y^RY^R. The failure to detect viable Y^RY^R females among sex reversals (X^rY^R ♀ ♀ + Y^RY^R ♀ ♀) estrogenized in two consecutive generations may be ascribed to the rare occurrence of developing Y^RY^R zygotes since the number of successful testcrosses was only 21 (16 *ER* ♀ > *ER* ♀ plus 5 *SR* ♀ > *SR* ♀).

In normal breeding of white females (X^rX^r) with heterozygous orange-red males (X^RY^r) of the medaka, in which was displayed a crisscross inheritance, AIDA (1936) obtained two exceptional white daughters having the genotype X^rY^r resulting from genic imbalance of sex genes. These exceptional X^rY^r females were mated with normal X^RY^r males. The observed proportion of white sons (X^rY^r ♂ ♂ + Y^rY^r ♂ ♂) to orange-red sons (X^RY^r ♂ ♂) to orange-red daughters (X^RX^r ♀ ♀) did not depart from that of the theoretical 2:1:1 ratio indicating that the Y^rY^r zygotes were as viable as X^rY^r and X^RY^r males. By testcrosses, he proved that about one half of white sons were Y^rY^r males. This is in strong contrast to the rarity of viable Y^RY^R zygotes demonstrated in our previous and present experiments. In this connection it is worth while noting that in the guppy (*Lebistes reticulatus*) WINGE (1934) demonstrated that $Y^{Ma}Y^{Pa}$ (*Ma*=maculatus. *Pa*=pauper) were viable and fertile, while WINGE and DITLEVSEN (1938, 1947) showed that $Y^{Ma}Y^{Ma}$ males are lethal. They postulated the presence of a lethal factor closely linked to the *Ma* gene.

GORDON (1946, 1947) demonstrated that "wild" stocks of the platyfish (*Platypoecilus maculatus*) from Mexico had the genetic mechanism for sex determination in which XX=female and XY=male—just opposite to the established type (WZ=female and ZZ=male) for the common aquarium-bred stock of the same species. GORDON (1951) reported that the platyfish from British Honduras belongs to the latter type (WZ-ZZ). CASTLE (1936) suggested that WZ and ZZ could be expressed as XY and YY, respectively. GORDON (1947) commented that although the W of the "domesticated" stock is not homologous to any X of the platyfish, the Z and the Y are apparently homologous. Whole populations of the female-heterogametic platyfish exist in which ZZ(YY) males represent the normal condition. Our finding that viable Y^RY^R males are rare in the medaka, where normal male is heterogametic (XY), may be interesting in view of evolution of heterosomes in fishes.

The lethality of the majority of Y^RY^R males in the medaka cannot be ascribed to their rapid energy consumption, although it might result in a relatively shorter life span. The present study indicates that if Y^RY^R females were ever viable, they also would be rare in spite of the fact that the female consumes less energy than the male. On the other hand, there is some evidence suggesting the presence of an

inert section in the ordinary Y^R chromosome, which causes lethality of the zygote homozygous for it. It is inferred that both the regular X^r and X^R contain a positive section, essential for viability, in the region corresponding to the inert section of the ordinary Y^R. The rare viable Y^RY^R male was found to be heterozygous for the inert section, *viz.*, one Y^R having the positive section derived from the X^r by crossing over (unpublished). Incidentally, the assumption of the presence of an inert section in the ordinary Y^R chromosome rationalizes readily the previously mentioned discrepancy between the observed (2♀:1♂) and expected (1♀:1♂) sex ratios among offspring of crossover orange-red females denoted by $X^rX^R_c$ mated with normal males (X^rY^R). The recombinant X^R_c is suspected to have received the inert section together with the *R* from the ordinary Y^R in crossing over occurring in the parents (sex-reversal X^rY^R ♀ and X^rY^R ♂). Hence, the $X^R_cY^R$ zygotes may be lethal because they are homozygous for the inert section. A detailed account on this problem is reserved until data from current experiments are completed.

The occurrence of WW females in cases of female heterogamety has also been recorded. Breider (1942), in breeding the domesticated platyfish (*Platypoecilus maculatus*), found an exceptional WZ male. This exceptional male was presumed to have the constitution $W^{RSF}Z^{Dr}$, and in a mating with a normal female W^+Z^R, sired 51 daughters and 13 sons. Among the daughters, he claimed to have obtained some WW females. This was highly probable but unfortunately testcrosses for detecting WW females were not carried out. If the WW females had been mated with normal males (ZZ), all female progenies would surely have resulted. Bellamy and Queal (1951), in breeding the same fish, found a number of exceptional WZ males which, in mating with normal WZ females, fathered WW females in addition to ordinary females (WZ) and males (ZZ). Progenies of these WW females by normal males (ZZ) consisted entirely of females. It was noted by them that WW females were less fertile. Humphrey (1945) proved experimentally that in ambystomid salamanders (*Siredon mexicanum*, *Ambystoma tigrinum*) WW females were viable and fertile. It is remarkable that Humphrey (1948) was able to show that males having the genotype WW, induced by grafting of testis preprimordium on WW embryos, are viable. These results led him to conclude that in these species the W chromosome is not greatly different from the Z except as to the sex-determining factor.

Data on offspring of sex reversals in the present study indicate that the percentage of both exceptional white males and exceptional orange-red females is significantly greater than that in the stock from which experimental fish were derived. This is particularly true with the offspring of sex reversals in two consecutive generations. That seven out of 503 or 1.4 percent of white fish are exceptional males and 35 out of 1,047 or 3.3 percent of orange-red fish are exceptional females, is shown in Table 3, fourth line. The frequency of exceptional fish in these data is 5–6 times that among the stock bred for several generations. In the stock exceptional orange-red females always exceed exceptional white males. The preponderance of exceptions among offspring of sex reversals over those among

the controls and the stock suggests the occurrence of crossing over between the X^r and Y^R chromosomes in both parents, *i.e.*, sex-reversal mothers (X^rY^R) and normal X^rY^R fathers.

SUMMARY

This study is on artificial induction of functional sex reversal in genotypic males (XY) of the medaka (*Oryzias latipes*) made under the influence of estrogens (estrone and stilbestrol) using the offspring of X^rX^r females mated to X^rY^R males (r=white, R=orange-red).

The continuous administration *per os* of either estrone or stilbestrol at the dosage level of 1,250 i.u./g diet, beginning with the time of hatching and ending at certain juvenile stages, covering the period prior to and passing through the stage of the gonadal sex differentiation, is sufficient to induce permanent sex reversal in the majority of genotypic males (X^rY^R). A rare intersex of the male genotype was also induced.

A single Y^RY^R male, the second ever to have been detected in our series of experiments, was detected among ten normal-fed F_1 sons of estrone-sex-reversals by X^rY^R males.

Functional sex reversal in genotypic males by means of estrogens was successful in two consecutive generations. So far, 16 estrone-sex-reversals and five stilbestrol-sex-reversals estrogenized for two consecutive generations and presumably having the genotype either X^rY^R or Y^RY^R, were singly tested to normal X^rY^R males. All those tested were found to have the genotype X^rY^R. No Y^RY^R females were detected, a fact ascribed to the rarity of surviving Y^RY^R zygotes.

The exceptions among offspring of sex reversals were proved to be either crossovers (X^rY^r males and X^rX^R females) or X^rY^R females arising from genic imbalance of sex genes. The preponderance of exceptions among offspring of sex reversals over those among controls and the stock suggests the occurrence of crossing over between the X^r and Y^R in both parents, *i.e.*, sex-reversed mothers (X^rY^R) and normal X^rY^R fathers.

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